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Habitat use, diet and roost selection by the Big Brown Bat (*Eptesicus fuscus*) in North America: a case for conserving an abundant species

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ABSTRACT

Insectivorous bats are integral components of terrestrial ecosystems. Despite this, a growing number of factors causing world-wide declines in bat populations have been identified. Relatively abundant species are important for bat conservation because of their role in ecosystems and the research opportunities they offer. In addition, species that have been well-studied present unique opportunities to synthesize information and highlight important areas of focus for conservation and research. This paper focuses on a well-studied abundant bat, *Eptesicus fuscus*. I review the relevant literature on habitat use, diet and roost selection by *E. fuscus* in North America, and highlight important areas of conservation and research for this species, including the effects of roost disturbance, control of economically important insect pests, exposure to pesticides, long-term monitoring of populations, and the potential consequences of expanding populations. These issues have broad implications for other species and can be used to focus future research and conservation efforts.

Keywords: bat conservation, *Eptesicus fuscus*, food habits, North America, research needs, roosts

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INTRODUCTION

Bat populations are declining world-wide as a result of a growing number of factors, including habitat loss and fragmentation, disturbances to roosts, exposure to toxins, human hunting pressures and introduced predators (McCracken, 1989; Fenton, 1997; Arita & Ortega, 1998; Fenton & Rautenbach, 1998; Marinho-Filo & Sazima, 1998; Pierson, 1998; Racey, 1998; Rainey, 1998; Richards & Hall, 1998; Utzurrum, 1998; O'Donnell, 2000). This makes it difficult to draw general conclusions about bat conservation, which may require species-specific conservation plans (Fenton, 1997). Insectivorous bats are major consumers of nocturnal insects, many of which are economically important pests. This presents both ecological and economic rationales for their protection (Grinnell, 1918; Constantine, 1970; Whitaker, 1995; Pierson, 1998). In addition, bat guano is rich in nitrogen and other nutrients. Bats may transfer significant amounts of nutrients in ecosystems as guano accumulates at roosts (e.g. tree hollows; Kunz, 1982; Rainey *et al*., 1992; Zielinski & Gellman, 1999) and is spread across the landscape while bats forage (Pierson, 1998). Bats are also important components of cave environments, where the accumulation of guano supports a diverse invertebrate community (Poulson, 1972; Culver *et al*., 2000). Some bat assemblages may be useful indicators of habitat disturbance and quality (Fenton *et al*., 1992; Medellin, Equihua & Amin, 2000).

Like most conservation efforts in North America, bat conservation has focused primarily on rare and endangered taxa (Pierson, 1998). However, because of their potential role in controlling insect populations and distributing nutrients across landscapes, Pierson (1998: 318) argued that widespread, abundant, species may be the most ecologically and economically important. In the UK, recent attention has been directed towards a national landscape-level bat conservation and management plan (Racey, 1998). The broad strategies gleaned from this effort have centred mainly around data collected from the Common Pipistrelle (*Pipistrellus pipistrellus*), one of the most widespread and abundant bats in Europe (Racey, 1998). This work illustrates the importance of abundant species, not only because of their numerical abundance and ecological impact, but also because of the research opportunities they present. In North America, several of the most abundant bats (e.g. *Eptesicus fuscus* and *Myotis lucifugus*) readily roost in buildings and artificial bat boxes (Tuttle & Hensley, 2000), presenting a practical means for ensuring their continued abundance.

Fenton (1997) and Pierson (1998) identified several components of bat conservation. These include (i) protection of foraging habitat; (ii) protection of the prey base; and (iii) protection of roosts. The objective of this paper is to review the relevant literature on habitat use, diet and roost selection by a relatively abundant bat species, *E. fuscus* (Chiroptera: Vespertilionidae), in North America. I focus on these broad components of bat conservation, using a well-studied species to illustrate the importance of species-specific information for determining conservation goals. In addition, I address the importance of conserving abundant bat species, because of both their role in ecosystems and the research opportunities they present. Finally, I identify some specific areas of research that relate directly to the conservation of *E. fuscus* and more broadly to bats in general.

THE BIG BROWN BAT

The Big Brown Bat (*Eptesicus fuscus*) is one of the most widespread mammals in North America, ranging from Canada throughout the United States and Central America, and into north-western South America (Kurta & Baker, 1990). It also occurs on several islands, including Cuba, Jamaica and Puerto Rico. This bat is the only North American representative of the genus *Eptesicus* north of Mexico, and probably has been widespread throughout the Pleistocene (Kurta & Baker, 1990). *Eptesicus fuscus* exhibits significant morphological variation across its range (Burnett, 1983) and is represented by 11 subspecies (Kurta & Baker, 1990). Across its range, it is distinguished from sympatric species by its relatively large size (14–30 g; Nowak, 1999), bi-coloured pelage (blackish-brown to pinkish-tan above, paler underneath), short blunt tragus and long fur (Kurta & Baker, 1990). Because of its widespread distribution and relatively high abundance, *E. fuscus* may play a particularly important role in many ecosystems. Compared with other species, *E. fuscus* has been well-studied (Kurta & Baker, 1990). This reflects its colonial behaviour and close association with humans (Davis, Barbour & Hassell, 1968; Barbour & Davis, 1969).

HABITAT USE

For many species, bat–habitat relationships are poorly understood. Several factors complicate this relationship, including the high mobility of bats, which gives them access to a wide range of habitats (Fenton, 1997). Recent advances in radio-tracking and bat-detector technology have allowed for significant progress in our understanding of bat–habitat relationships (Fenton, 1997). The UK National Bat Habitat Survey, for example, has developed important generalizations and produced powerful predictive equations regarding habitat use by bats at local and landscape levels (Walsh & Harris, 1996a, 1996b).

Big Brown Bat habitat associations

Studies of *E. fuscus* in North America have failed to establish unique associations with specific habitats (Bell, 1980; Geggie & Fenton, 1985; Furlonger, Dewar & Fenton, 1987; Krusic & Neefus, 1996) and suggest that this bat is a habitat generalist (Furlonger *et al.*, 1987; Krusic & Neefus, 1996). No clear associations are documented between city, town and rural settings (Geggie & Fenton, 1985; Furlonger *et al.*, 1987) or between forest types (Bell, 1980; Krusic & Neefus, 1996). Some habitat features appear to be important to *E. fuscus* when foraging. In the White Mountains of New Hampshire, Krusic & Neefus (1996) found that the activity of *E. fuscus* was highest near standing water and roads. In Arizona, Bell (1980) observed higher activity in riparian zones. In topographically diverse regions, foraging activity by reproductive females appears to be greater at lower elevations where insect densities are higher (Cryan, Bogan & Altenbach, 2000). Foraging activity has also been shown to decrease with increasing urbanization, possibly because of lower insect abundance in these areas (Geggie & Fenton, 1985).

Habitat is probably a less important conservation component for *E. fuscus* than for other species, although current forestry practices may exert a negative impact on some tree-roosting populations (Betts, 1996; Vonhoff, 1996; Vonhoff & Barclay, 1996; Kalcounis & Brigham, 1998; Rabe *et al.*, 1998). *Eptesicus fuscus* readily takes advantage of insect concentrations near lights (Geggie & Fenton, 1985; Furlonger *et al.*, 1987) and readily uses human-made structures as roosts (Whitaker & Gummer, 1992, 2000; Williams & Brittingham, 1997). These two behaviours have probably lessened any potential impacts of habitat loss on *E. fuscus*. Several factors related to diet and roost selection, however, may confound

the otherwise neutral (or positive; Whitaker & Gummer, 1992; Fenton, 1997) impacts that human modification of the environment has had on this species.

THE PREY BASE

Insectivorous bats are susceptible to the accumulation of toxins (e.g. pesticides) because of their high trophic rank and longevity (Clark, 1988). Knowledge of the food habitats of bats is useful for identifying potential sources of toxins (Clawson & Clark, 1989). In addition, knowledge of food habits enables the identification of agricultural pests consumed by bats (Whitaker, 1995) and publicizing this information can be a powerful conservation tool. These two issues (exposure to pesticides and consumption of insect pests) are closely linked, and both are important when considering the conservation of bats.

Food habits

A number of studies in the US and Canada have examined the food habits of *E. fuscus*; however, studies on more southern populations are generally lacking (Table 1). Black (1974) classified *E. fuscus* as a beetle-strategist (predator of Coleoptera) in New Mexico; the current literature appears to support this, with a few notable exceptions. Studies in Arizona (Warner, 1985) and Oregon (Whitaker, Maser & Keller, 1977; Whitaker, Maser & Cross, 1981) have found moths (Lepidoptera) to be major prey items, although moths are generally minor com-

Table 1. Summary of *Eptesicus fuscus* food habits in North America

Location	Method*	Dominant prey items	Second major prey items	Source
Indiana, Illinois	%v	Coleoptera: Scarabaeidae, *Diabrotica*	Hemiptera: Pentatomidae	Whitaker (1995)
Indiana	%v	Coleoptera: Carabidae, Scarabaeidae, *Diabrotica*	Hemiptera: Pentatomidae	Whitaker (1972)
Oregon	%v	Lepidoptera	Coleoptera: Scarabaeidae	Whitaker *et al.* (1977)
	%v	Coleoptera: Scarabaeidae, Carabidae	Lepidoptera	Whitaker *et al.* (1981)
	%v, %f	Trichoptera	Coleoptera: Scarabaeidae	Verts *et al.* (1999)
New Mexico	%f	Coleoptera	Not applicable	Black (1974)
Arizona	%f	Lepidoptera	Coleoptera	Warner (1985)
West Virginia	%f	Coleoptera: Scarabaeidae	Hymenoptera	Hamilton (1933)
Kansas	%v	Coleoptera: Scarabaeidae, Carabidae	Hemiptera: Pentatomidae	Phillips (1966)
Maryland	%f	Coleoptera	Hemiptera: Pentatomidae	Griffith & Gates (1985)
British Columbia	%a	Trichoptera	Diptera	Brigham (1990)
	%a	Trichoptera	Diptera, Coleoptera	Brigham & Fenton (1991)
Alberta	%v	Coleoptera	Hemiptera	Brigham & Saunders (1990)
	%v	Coleoptera	Hemiptera, Lepidoptera†, Diptera†	Hamilton & Barclay (1998)

*%v = percentage volume of prey type in faecal or stomach sample; %f = percentage frequency of occurrence of prey type; %a = percentage abundance of prey type.
†Second major prey items in the second year of the study.

ponents of the diet (Hamilton, 1933; Ross, 1967; Black, 1972, 1974; Whitaker, 1972, 1995; Griffith & Gates, 1985; Brigham & Saunders, 1990; Hamilton & Barclay, 1998). In parts of British Columbia and Oregon the dominant prey of *E. fuscus* appears to be large caddisflies (Trichoptera), whereas beetles are relatively unimportant (Brigham, 1990; Brigham & Fenton, 1991; Verts, Carraway & Whitaker, 1999). It should be noted that dietary studies are often limited temporally (e.g. Verts *et al*.'s 1999 data were restricted to July), which may bias conclusions on overall diet in an area.

The diets of most insectivorous bats probably reflect temporal, seasonal and geographical variation in insect abundance, with some degree of flexibility in prey selection (Kunz, 1974a; Anthony & Kunz, 1977; Jones, 1990; Whitaker, 1995; Whitaker, Neefus & Kunz, 1996). *Eptesicus fuscus* has large, powerful, jaws (Freeman, 1981) and preys mainly on beetles and other hard-bodied insects (e.g. Hemipterans; Table 1; S. J. Agosta & D. Morton, unpublished data from Pennsylvania and Maryland) in regions that have been studied. However, this bat can exploit a variety of other prey types and is flexible both temporally and spatially with regard to prey use (Brigham, 1991; Whitaker, 1995; Hamilton & Barclay, 1998; S. J. Agosta & D. Morton, unpublished data).

Whitaker (1995) did the most extensive study of the food habits of *E. fuscus*, examining variation among and within maternity colonies in Indiana and Illinois. Significant variation in diet existed among and within colonies, but beetles and stink bugs (Hemiptera: Pentatomidae) comprised the majority of prey. A number of food items found by Whitaker (1995) and others (Table 1) are important agricultural pests (Table 2). Estimates of the actual numbers of these pests consumed annually by one mid-western *E. fuscu*s colony are substantial (Table 2), and the potential utility of this bat as a biological control agent for harmful insects has been emphasized (Whitaker, 1993, 1995).

Pesticides

Currently, pesticides are the primary means of controlling agricultural pests, which undoubtedly places wildlife at risk of chemical exposure (Smith, 1987; McLaughlin & Mineau, 1995). Pesticides have a variety of effects on *E. fuscus* and other bat species. These include direct mortality (Clark, Laval & Krynitsky, 1980; Clark, 1981; Clark, Clawson & Stanford, 1983), altered behaviour (Clark, 1986; Clark & Rattner, 1987) and transfer of toxins to nursing

Table 2. Agricultural pests commonly preyed on by *Eptesicus fuscus*

Pest	Common name	Estimated number consumed by a mid-western colony of 150 bats/year‡	Some crops damaged§
Chyrsomelidae			
Diabrotica			
Adults	Cucumber beetles	600 000	Cucumbers, other cucurbits, corn
Larvae*	Rootworms	33,000,000	
Pentatomidae†	Stink bugs	335 000	Soybean, cotton
Scarabaeidae	Scarab beetles	194 000	Various crops, lawns and nurseries
Cicadellidae	Leafhoppers	158 000	Various crops, including potato, apple and corn

*Secondary effect of preying on adult females.
†Mainly the Green Stink Bug (*Acrosternum hilare*).
Sources: ‡Whitaker (1995); § Davidson & Lyon (1987).

young (Clark & Lamont, 1976). The adverse effects of organochlorine pesticides (e.g. DDTs) on bats have been well-documented (Jefferies, 1972; Clark, 1981, 1988). In the US, organochlorines have been banned and replaced with organophosphate and carbamate pesticides, although organochlorine residues still persist in soils and still accumulate in some bat populations (Thies, Thies & McBee, 1996).

Organophosphate and carbamate pesticides are expected to be less toxic than organochlorines (Smith, 1987; Clark, 1988); however, some currently used pesticides reportedly cause mortality in birds and other mammals (Grue *et al*., 1983; Smith, 1987; Augspurger *et al*., 1996). Pesticide exposure may be an important cause of decline for some populations of insectivorous bats (Jefferies, 1972; Reidinger, 1972), particularly species whose diet includes a substantial portion of agricultural pests. McCracken (1989) concluded that pesticides are usually not a major factor in the decline of bats, and emphasized the role of roost disturbance (see below). Despite this, little field research has been conducted on the levels of exposure or the sublethal effects of these chemicals on bats (but see Swanepoel *et al*., 1999). In addition, few studies have attempted to link pesticide exposure to specific insect prey or specific habitats where bats are foraging (Clawson & Clark, 1989). Research is also needed to address the indirect effects of pesticide use in habitats where bats forage, particularly the potential for overall reductions of the prey base.

ROOST SELECTION

Roost selection by bats has implications for a variety of life-history traits and is vital for survival and reproduction (Kunz, 1982; Tuttle & Stevenson, 1982). Roost selection often varies seasonally and roosts serve a number of functions (reviewed by Kunz, 1982). For many temperate bats, these can be separated into winter hibernacula, maternity roosts and summer roosts (males and non-reproductive females). Selection of suitable roosts is important for growth, development and survival of young (Tuttle, 1975; Tuttle & Stevenson, 1982), protection from predators (Fenton, 1983), protection from the elements (Vaughan, 1987), and reduction of thermoregulatory costs (Kurta, 1985). In addition, many bats use specific night roosts in close proximity to foraging areas (Kunz, 1982). Night roosts may function as resting places that facilitate digestion between feeding bouts and may provide opportunities for social interactions (Kunz, 1982). Thus, it is important to understand the roosting requirements of bats to ensure adequate roost protection and availability. In general, protection of only one roost type is not adequate and temporal variation in roost selection must be accounted for when determining conservation goals (Fenton, 1997; Pierson, 1998).

Eptesicus fuscus roosts in a wide variety of structures. These include caves, tunnels and mines (Rysgaard, 1942; Twente, 1955; Beer & Richards, 1956; Mumford, 1958; Phillips, 1966; Mills, Barrett & Farrell, 1975; Gates *et al*., 1984; Dalton, 1987; Raesly & Gates, 1987), buildings (Whelden, 1941; Davis *et al*., 1968; Brigham & Fenton, 1986; Williams & Brittingham, 1997; Whitaker & Gummer, 2000), bat boxes (Brittingham & Williams, 2000; Tuttle & Hensley, 2000) and tree cavities (Table 4). Roosts also have been located in rock crevices (Brigham, 1988), storm sewers (Goehring, 1972) and wood piles (Mills *et al*., 1975). Most observations of *E. fuscus* roosts have come from studies that have not focused specifically on roost selection. A few studies have examined roost selection by comparing occupied vs. unoccupied sites (Table 3 and see below; for factors influencing tree-roost selection see Betts, 1996; Vonhoff, 1996; Vonhoff & Barclay, 1996; Kalcounis & Brigham, 1998; Rabe *et al*., 1998). Such studies are necessary to understand roost selection by bats fully, especially when the goal is to develop useful conservation strategies (Crampton & Barclay, 1998).

Buildings, caves and mines

Raesly & Gates (1987) examined winter roost selection in caves and mines by several species of bats in the north-eastern US. Factors that influenced site selection by *E. fuscus* are summarized in Table 3. Among available hibernacula, *E. fuscus* selected larger caves and mines with relatively high airflow. Within hibernacula, *E. fuscus* was a solitary hibernator (but may form small clusters; Rysgaard, 1942; Mumford, 1958; Phillips, 1966; Whitaker & Gummer, 1992) that occupied relatively cool, dry cave walls in areas with noticeable airflow. Rysgaard (1942) observed similar conditions among hibernacula in Minnesota. In buildings, selection of hibernacula may be correlated with the presence of heating that maintains temperatures above freezing (Whitaker & Gummer, 1992, 2000). Buildings otherwise suitable for maternity colonies are not always utilized as hibernacula and vice versa (Whitaker & Gummer, 1992, 2000), a fact that further complicates roost protection.

Eptesicus fuscus primarily forms maternity colonies in buildings (Davis *et al*., 1968; Barbour & Davis, 1969; Mills *et al*., 1975; Whitaker & Gummer, 1992, 2000; Williams & Brittingham, 1997) but also in tree cavities (Table 4). Williams & Brittingham (1997) examined factors influencing the selection of buildings by *E. fuscus* in Pennsylvania. Important site-selection variables are summarized in Table 3. Maternity roosts were typically present in older buildings with numerous access points (see also Schowalter & Gunson, 1979; Brigham & Fenton, 1986, 1987). Occupied buildings exhibited higher daytime temperatures and wider temperature gradients than unoccupied buildings. Roost temperature is important for growth and development (Tuttle, 1975; Tuttle & Stevenson, 1982) and it is hypothesized that bats select roosts to take advantage of factors that enhance reproductive success (Brigham & Fenton, 1986; Williams & Brittingham, 1997).

Table 3. Habitat characteristics important to roost selection by *Eptesicus fuscus*

Roost type/structure	Important habitat variables§	Location
Hibernacula/cave*	Entrance area	Maryland, Pennsylvania, West Virginia
	Average passage height	
	Maximum passage height	
	Airflow	
	Number of entrances	
	Minimum ambient temperature (–)¶	
	Maximum ambient temperature (–)	
	Maximum wall temperature (–)	
	Minimum relative humidity (–)	
	% standing water (1 km^2 radius)	
Maternity/building†	Number of access points	Pennsylvania
	Building age	
	Attic height	
	Roof material (tin/steel)	
	Maximum daytime temperature	
	Temperature gradient	
	% surrounding agriculture	
Hibernacula/building‡	Heated attic**	Indiana, Illinois
	Maintenance of temperature above freezing**	

Sources: Raesly & Gates (1987)*; Williams & Brittingham (1997)†; Whitaker & Gummer (1992)‡.
§Variables were considered important if significantly different from unoccupied sites ($P < 0.05$).
¶(–), variable less than that of unoccupied sites.
**Did not perform statistical analyses.

Roost site selection by male and non-reproductive female *E. fuscus* is not constrained by the costs of reproduction, and they are typically not associated with maternity colonies (Mills *et al*., 1975; Hamilton & Barclay, 1994); although males may occupy separate portions of maternity roosts or gradually move into maternity roosts as the young become weaned (Davis *et al*., 1968). Selection of summer roosts by *E. fuscus* has received little attention, probably because aggregations are often small and dispersed (Barbour & Davis, 1969). It is expected that, because males and non-reproductive females are not tied to maternity roosts, they select cooler roosts that facilitate entry into torpor (Hamilton & Barclay, 1994; Grinevitch, Holroyd & Barclay, 1995). Summer roosts have been found in caves and abandoned mines (Phillips, 1966) and a variety of other structures, including buildings, shutters and wood piles (Mills *et al*., 1975). Recently, bridges have been implicated as important night roosts for both male and female *E. fuscus* in the western US (Pierson, Rainey & Miller, 1996; Adam & Hayes, 2000). In the eastern US, *E. fuscus* reportedly uses caves (Davis *et al*., 1968) and mines (Agosta, Kuhn & Morton, in press) as night roosts.

Tree cavities

Although often referred to as a cave bat, *E. fuscus* also utilizes tree cavities in some regions (Table 4). Tree-roosting *E. fuscus*, primarily maternity colonies, occur mainly in the western US and Canada (Table 4). However, the current distribution of tree-roosting populations may reflect a bias in study objectives and methods (e.g. radio-tracking individuals). Brigham (1991) studied eight *E. fuscus* maternity colonies in British Columbia that primarily occupied tree cavities. This suggests that the availability of tree cavities is important to some populations. In parts of Saskatchewan, *E. fuscus* is a secondary cavity rooster, occupying Trembling Aspens (*Populus tremuloides*) excavated by Sapsuckers (*Sphyrapicus varius*) (Kalcounis & Brigham, 1998). They concluded that aspen cavities may be a limiting resource for *E. fuscus* in Saskatchewan.

Historically, *E. fuscus* probably formed maternity colonies exclusively in tree cavities (Whitaker & Gummer, 1992; Williams & Brittingham, 1997). More recently, the incidence of tree-roosting behaviour may be interpreted either as a preference for natural roosts where they are available or the use of natural roosts where buildings are not abundant (Brigham, 1991). Human development may actually have decreased the relative importance of natural roosts in regions where buildings are abundant and offer relatively large, permanent, structures. Higher fidelity by *E. fuscus* to buildings than to tree cavities (Brigham, 1991) suggests that buildings offer some advantages. Buildings may often be more abundant than tree cavi-

Table 4. Tree–roost associations of *Eptesicus fuscus* in North America

Location	Tree species	Roost type	Source
British Columbia	*Pinus ponderosa*	Maternity	Brigham (1991), Vonhoff (1996)
	Thuja plicata	Maternity	Vonhoff & Barclay (1996)
	Populus tremuloides	Maternity	Vonhoff (1996)
	Psuedotsuga menziesii	Maternity	Vonhoff (1996)
Saskatchewan	*Populus tremuloides*	Maternity	Kalcounis & Brigham (1998)
Arizona	*Pinus ponderosa*	Maternity	Rabe *et al*. (1998)
Oregon	*Pinus ponderosa*, *Populus trichocarpa*	Maternity	Betts (1996)
California	*Sequoia sempervirans*	Hibernacula	Rainey *et al*. (1992)
Maryland	*Quercus* spp.	Maternity	Christian (1956)
Michigan	*Fagus grandifolia*	Maternity	Kurta (1980)

ties near preferred habitat features (e.g. lights, water and roads) and may offer more stable microclimates.

More work is needed on tree-roost selection by *E. fuscus* to warrant a discussion on the importance of these roosts relative to building roosts (Brigham, 1991); however, previous authors discuss some important management implications (Vonhoff & Barclay, 1996; Kalcounis & Brigham, 1998). *Eptesicus fuscus* has been found roosting in trees in Michigan (Kurta, 1980) and Maryland (Christian, 1956), suggesting that this behaviour is more prevalent in the eastern US than the current literature indicates. Radio-tracking studies of *E. fuscus* in the eastern US are needed. The remainder of this discussion focuses on building-, cave- and mine-roosting populations, while acknowledging that natural tree cavities are an important component of the roosting ecology of *E. fuscus*.

Human impacts to roosts

Bats roosting in buildings, caves and mines are particularly vulnerable to human disturbance and exclusion. Human disturbance to roosts, including the activities of researchers, can have deleterious effects on resident bat populations (Mohr, 1972; Reidinger, 1972; Tuttle & Stevenson, 1982; McCracken, 1989). For example, Tuttle (1975) reported that disturbances to Gray Bat (*Myotis grisescens*) maternity colonies can result in heavy mortality of the young, who may be abandoned by fleeing females. Reidinger (1972) attributed declines in several Arizona bat populations partly to human disturbances at roosts. Recently, Thomas (1995) has shown that increased flight activity by hibernating bats occurs subsequent to human presence, which may cause premature depletion of fat reserves and increased winter mortality. This potentially important source of mortality requires more study, particularly because researchers often conduct population censuses when bats are highly aggregated in hibernacula.

Although many natural caves and mines are now protected (e.g. gated and fenced), unauthorized visitation still occurs and the effects of these disturbances have not been properly assessed in most situations. Culver *et al*. (2000) have even suggested that current methods of cave gating, while providing protection for bats, may have negative impacts on other cave fauna. Many obligate cave fauna in the US are considered vulnerable or threatened (e.g. > 95% of the terrestrial and aquatic species). Evidence that current methods of cave gating negatively impact these species may create a need for new solutions that provide protection for a broader array of cave fauna, not only bats (Culver *et al*., 2000). Bats roosting in caves and mines are also vulnerable to environmental disturbance (e.g. floods and structural collapse). With some foresight, structural collapse and floods may be avoided, although providing protection for all roosts is probably not feasible. Caves and mines supporting large populations or high species diversity should be assessed at a state-wide level and given special concern (Gates *et al*., 1984; Dalton, 1987; Arita, 1996).

Bats that roost in buildings are often perceived as a nuisance and are vulnerable to exclusion and eradication attempts (Brigham & Fenton, 1986, 1987; Neilson & Fenton, 1994; Williams & Brittingham, 1997; Brittingham & Williams, 2000). Little information exists on the effects of the displacement of bats from buildings on their reproductive and survival success. Radio-tracking has shown that *E. fuscus* excluded from buildings readily moves to nearby buildings, but that reproductive output may be reduced (Brigham & Fenton, 1986, 1987). Goehring (1972) observed an increase in a population of *E. fuscus* roosting in a sewer that coincided with the removal of old buildings in the area. Neilson & Fenton (1994) banded 547 Little Brown Myotis (*M. lucifugus*) prior to exclusion from buildings. Only five individuals were found to relocate to nearby buildings, suggesting a significant decline in the local population. Assuming that attempts at exclusion from buildings are similar to disturbances

at caves and mines, the effects of these practices on bats may be expected to include reduced survival and reproduction. Proximate causes of these effects may include occupation of buildings with less desirable microclimates and greater distances to water and foraging areas.

SYNTHESIS

Conservation implications

As theory in conservation science shifts from a single species or closed system approach to an ecosystems approach (Minta, Kareiva & Curlee, 1999), the importance of abundant species becomes clearer. Insectivorous bats, as a group, are primary insect consumers. In this context, abundant species (e.g. *E. fuscus*, *M. lucifugus* and *Tadarida brasiliensis* in North America) probably play critical ecosystem roles (Pierson, 1998). Therefore, while in practice conservation efforts may continue to focus on rare and endangered species, relatively abundant species should be considered important for bat conservation as a whole. Continuing research to identify sources of population declines and important life-history requirements of abundant bats, so defining their conservation needs, should be useful in directing research for other species. In addition, preserving the continued abundance of abundant bats, in an otherwise declining group of mammals, is consistent with an ecosystems approach to conservation.

What lessons concerning bat conservation can we learn from the well-studied Big Brown Bat? First, this species illustrates the difficulty of applying dietary information from one area to unstudied areas. This may be particularly important when trying to monitor the prey base for potential sources of toxins or when trying to determine the extent of predation on agricultural pests. For example, the vast majority of food habits studies suggest that beetles, particularly scarab beetles (Scarabaeidae), are the major prey of *E. fuscus* throughout its range (Table 1). However, in certain areas beetles appear to be relatively unimportant, whereas large caddisflies are (Table 1). Vaughan (1997) reviewed the diets of British bats and observed that many species exhibit geographical variation in diet, but she concluded that the source of this variability was unclear. For *E. fuscus*, it is possible that either a temporal (e.g. seasonal and yearly) or spatial component is the key factor in the disparity between dietary studies. It may be significant, however, that studies where caddisflies were the dominant prey are restricted to the north-western portion of its range (Table 1). Are *E. fuscus* populations that feed primarily on caddisflies less susceptible to pesticide exposure than, for example, the mid-western colony cited in Table 2?

Secondly, a review of roost use and selection by *E. fuscus* illustrates the difficulty of providing adequate roost protection for bats. A threatened population may require simultaneous protection of a maternity roost, a variety of summer day roosts, a variety of summer night roosts, and a number of hibernacula that may or may not be different from the maternity roost. In Indiana, Whitaker & Gummer (2000) estimated that a single maternity colony of 150 *E. fuscus* will disperse into about 85 building hibernacula. Protecting roosts is further complicated by the fact that maternity roosts and hibernacula are often located in buildings that are privately owned, and the remaining roost types are difficult to locate. For *E. fuscus* and other species associated with humans, local and regional initiative is needed to encourage the public to report bat roosts routinely to state agencies or local researchers. This can best be done with continued emphasis in the media on the importance of bats and their dependence on anthropogenic structures.

A further consideration is regional differences in the relative importance of roost types. Currently it appears that distinct regional differences exist in the selection of maternity roosts by *E. fuscus* (see above); however, more work is needed to determine the relative importance

of roost types in these regions (Brigham, 1991). If regional differences in roost selection do exist, caution must be taken when trying to apply information from one area to another. An important issue that needs to be addressed in the future is the degree to which populations and individuals exhibit plasticity in roost selection. Brigham (1991) compared two *E. fuscus* populations in British Columbia and Ontario and observed differences in roost structure and roost fidelity, indicating flexibility among populations. Unfortunately, long-term data are lacking on the reproductive and survival success of individual bats forced to exploit alternative roost types after former roost types or conditions become unavailable.

Future research

Several areas of research regarding the conservation and protection of *E. fuscus* populations in North America can be identified from this discussion. These should apply to other relatively abundant bat species and/or species with similar food or roost habits. In addition, the information gained from this research should allow for useful generalizations regarding bat conservation.

Effects of roost disturbance

More research is needed on the levels and effects of disturbance at *E. fuscus* roosts, particularly buildings that house maternity colonies. It is likely that many roosts have not been accounted for (Mills *et al*., 1975; Whitaker & Gummer, 2000) and that most disturbances have gone unnoticed. In addition, public concern about rabies continues to pose threats to bats roosting in anthropogenic structures, and attempts at exclusion are likely to continue. Often, buildings (and caves and mines) may act as ecological traps (*sensu* Hassinger, 1994; Pulliam, 1996), whereby they offer suitable roost characteristics but ultimately lead to population declines because of human activities (Hassinger, 1994). This issue should be addressed, particularly in the context of source–sink dynamics (Pulliam, 1996).

More work is also needed to determine the effects of exclusion in order to develop methods that minimize human–bat conflict (Brigham & Fenton, 1987) and maximize reproductive and survival success. One option is to encourage bats to occupy alternative roosts, such as bat boxes (Williams & Brittingham, 1997; Brittingham & Williams, 2000; Tuttle & Hensley, 2000). Success in encouraging evicted maternity colonies to occupy bat boxes has been variable (Neilson & Fenton, 1994). In Pennsylvania, Brittingham & Williams (2000) have demonstrated that *E. fuscus* and *M. lucifugus* maternity colonies excluded from buildings will move successfully to bat boxes, provided the boxes are in close proximity to previous roosts and offer suitable microclimates.

Biological control

More research is needed to address the role of *E. fuscus* and other bats as biological agents for controlling harmful insects (for a review of biological control see Waage & Mills, 1992). Efforts to quantify (rather than speculate about) the potential economic benefits of bats to the agricultural industry may lead to reductions in the use of pesticides and an increase in the acceptance of bats. Demonstrating and quantifying the credibility of bats as an alternative to some pesticides will take creative manipulative experiments, such as those applied to insectivorous birds (Holmes, Schultz & Nothnagle, 1979; Atlegrim, 1989). However, the benefits to be gained from such studies should be considered. The success of projects such as Bat Conservation International's North American Bat House Research Project (Tuttle & Hensley, 2000) are encouraging, and suggest that large populations of bats (notably *E. fuscus* and *M. lucifugus*) are readily established in a variety of settings. Similar success has been reported

with Brown Long-eared Bats (*Plecotus auritus*) in Europe (Boyd & Stebbings, 1989; Benzal, 1991).

Pesticides

More research is needed on the presence and levels of pesticides in bats, the presence and levels of pesticides in prey, and the effects of these pesticides on the reproduction and survival of both bats and their prey. The toxicity of different pesticides to wildlife is varied (Clark, 1981; Smith, 1987). This dictates a need for food habits studies that examine sources and types of pesticide exposure to bats. Once the important prey items are identified, efforts should be made to examine pesticide levels in insects sampled in potential foraging areas (Clawson & Clark, 1989). In addition, knowledge of the relationship between pesticide residues in bats captured at roosts and the proximity of roosts to known areas of pesticide use would be useful (Reidinger, 1972). Geographic information systems (GIS) have been used increasingly as a conservation tool and could be used to develop spatial models relating pesticide use in the surrounding landscape to risk of exposure to bats. For *E. fuscus* and other species that commonly form maternity colonies in anthropogenic structures, the well-documented detrimental effects of chemically treated wood on European bats (Racey & Swift, 1986; Mitchell-Jones *et al.*, 1989) should be a cause for concern and immediate research.

Autecological studies

Although well-studied compared with other species, more research is needed on the general ecology of *E. fuscus* throughout much of its extensive range. In addition, little attention has been given to the possibility of ecological variation between subspecies. From a conservation standpoint, more information is needed on diet and roost selection, particularly outside the US and Canada. Information on summer roost selection by male and non-reproductive female bats is practically non-existent, and factors influencing selection of night roosts are just beginning to be understood (Adam & Hayes, 2000). More information is especially needed on the relationship between prey selection and specific habitats where bats forage (J.O. Whitaker, personal communication), and on the foraging habitat preferences of bats at a landscape level (cf. Walsh & Harris, 1996a, 1996b).

Long-term population monitoring

Long-term monitoring of *E. fuscus* populations needs to be initiated or continued. Because this species is widespread, it can be found in areas impacted to varying degrees by humans. This presents the opportunity to assess the effects of various types of land use and disturbance on reproduction and survival by comparing long-term population trends. Care should be taken to design robust monitoring programmes, in which representative *E. fuscus* populations associated with different types of land use and degrees of disturbance are monitored at suitable spatial and temporal scales. Such monitoring programmes are essential to extrapolate population trends to larger scales and to make meaningful comparisons of population trends across different habitats (Gibbs, 2000). Comparing population trends under a variety of conditions (e.g. high vs. low pesticide-use areas) may help to determine what factors are limiting to *E. fuscus* populations; the factors limiting bat populations have been a long-standing question among bat biologists (Fenton, 1997).

Demographic data suitable for risk assessment

Long-term *E. fuscus* monitoring programmes should include the collection of demographic data suitable for models of risk assessment. Population viability analysis (PVA), for example,

has been used increasingly as a conservation tool to model the persistence (probability of extinction) of populations over specified periods of time, and to investigate the sensitivity of populations to changes in parameters that affect population persistence (Boyce, 1992; White, 2000). Demographic models of population persistence are often applied to small populations with some form of conservation status (e.g. endangered species). Demographic modelling may also be useful with species such as *E. fuscus* to make relative comparisons between the trajectories of populations associated with different types of land use and levels of disturbance.

A major problem with demographic modelling is obtaining empirical data to drive the models (i.e. the parameters of the model are often based on limited data or guess-work), resulting in many applications that are of little practical use for conservation and management (Beissinger & Westphal, 1998; White, 2000). Essential data needed to conduct demographic risk assessment, in which conservation and management decisions can be based, include at a minimum estimates of age-specific survivorship and fecundity. For these models to perform realistically, some estimate of spatial, temporal and individual variation in these parameters must also be available (White, 2000). Collecting data suitable for models of risk assessment thus requires long-term demographic studies at suitable spatial scales. However, conducting these studies on endangered, rare or small populations is often impossible. In such cases, White (2000) recommended using surrogate data from closely related species or species in similar ecological guilds. Although this recommendation referred to the use of long-term data sets available from game species, it can be extended to include data collected from readily studied, relatively abundant, species such as *E. fuscus*.

Currently, data suitable for demographic analysis do not exist for most bat species. One problem is that the structure and dynamics of bat populations are not well-understood (Fenton, 1997), although recent studies have elucidated the population structure of some species (Burland *et al*., 1999; Entwistle, Racey & Speakman, 2000). Entwistle *et al*. (2000), for example, found that colonies of *P. auritus* occupying bat boxes exhibited minimal immigration and emigration and high roost fidelity, which is consistent with a metapopulation model (Hanski & Gilpin, 1997). While good data on *E. fuscus* colony size (Whitaker & Gummer, 2000) and roost fidelity (Brigham, 1991) exist for some regions, little or no data exist on immigration and emigration. Estimates of *E. fuscus* survival rates (Beer, 1955; Goehring, 1972; Mills *et al*., 1975; Hitchcock, Keen & Kurta, 1984) and mean litter sizes (Kunz, 1974b) are available, although most studies do not include data on spatial or temporal variation in these parameters (but see Hitchcock *et al*., 1984). The available data also come from various locations at various points in time, which would reduce the reliability of demographic models applied to real populations.

Long-term *E. fuscus* monitoring programmes are therefore needed to (i) detect changes in abundance; (ii) relate population trends to various types of land-use; and (iii) collect demographic data suitable for modelling population persistence, both for *E. fuscus* and as surrogate data for other bat species. Parallel research is also needed to determine the structure and dynamics of *E. fuscus* populations at various scales. Currently, *E. fuscus* population structure is being investigated at a regional scale (A. Turmelle *et al.*, unpublished data), which should give valuable insights into the proper scale and design of monitoring programmes.

Expanding Big Brown Bat populations?

Finally, research is needed to address the possibility of expanding *E. fuscus* populations. Historically, the abundance of *E. fuscus* in the northern portion of its range may have been limited by the availability of suitable winter hibernacula (e.g. hibernacula that maintain

temperatures above freezing). Whitaker & Gummer (2000) suggested that *E. fuscus* populations are increasing in the northern portion of its range because of the availability of buildings with heated attics. One consequence of expanding *E. fuscus* populations may be competition with other bat species, particularly *M. lucifugus*, which often forms summer colonies in buildings (Whitaker & Gummer, 2000).

Competition for resources has been difficult to demonstrate with bats, primarily because experimental manipulations are extremely difficult (Findley, 1993). Researchers have documented evidence of competition between sibling bat species (Arlettaz, Perrin & Hausser, 1997) and of past competitive interactions that may have shaped some New World bat assemblages (Stevens & Willig, 1999). Recently, Arlettaz, Godat & Meyer (2000) found evidence of competition for food between *P. pipistrellus* and the Lesser Horseshoe Bat (*Rhinolophus hipposideros*) in Switzerland. They suggested that competition with expanding *P. pipistrellus* populations may contribute to dramatic declines of *R. hipposideros* in western Europe.

Comparative studies indicate little dietary overlap between *E. fuscus* and *M. lucifugus* (Whitaker, 1972; Whitaker *et al*., 1977, 1981; Griffith & Gates, 1985). If expanding *E. fuscus* populations are causing increased competitive interactions with *M. lucifugus*, competition for roosts, not food, seems likely. *Eptesicus fuscus* is twice the size of *M. lucifugus* (14–30 g and 5–14 g, respectively; Nowak, 1999) and direct (interference) competition within roosts would probably favour the larger species. Mills *et al*. (1975) observed the movement of *E. fuscus* into the attic of a church that was occupied by 600 *M. lucifugus*. After a year, the *E. fuscus* colony increased from 20 to 50 individuals while the *M. lucifugus* colony decreased by 75%. Roost sites previously occupied by *M. lucifugus*, but later occupied by *E. fuscus*, have also been reported (Cope, Whitaker & Gummer, 1991).

CONCLUSIONS

Eptesicus fuscus is unique in many North American bat assemblages in that it is often the most abundant species adapted to a hard-bodied diet (Freeman, 1981). As a result, it contributes the greatest level of consumption of certain insects, several of which are important agricultural pests. In addition, the ability of *E. fuscus* to take advantage of human-made structures as roosts and exploit a variety of foraging habitats has probably lessened any potential impact of habitat loss, and increased its abundance from historical levels (Whitaker & Gummer, 1992), as has the presence of human-induced prey concentrations (e.g. lights; Fenton, 1997). This may further the potential of this bat to be utilized as a biological agent for controlling economically important insect pests. However, this may be confounded by the fact that bats living in anthropogenic landscapes are subjected to a variety of pressures that may limit populations.

Currently, two issues complicate our ability to understand the conservation needs of bats. First, we have yet to define, unequivocally, the structure and dynamics of bat populations (i.e., what constitutes a population of bats), although important advances have been made by use of molecular genetics (Burland *et al*., 1999). Secondly, although we have a general idea of the factors negatively affecting bats (e.g. roost disturbance, pesticide exposure, habitat loss, etc.), the natural history of many species is poorly understood. Without specific information on habitat use, roost selection and diet, and how these vary over space and time, it is difficult to draw conclusions regarding species-specific conservation needs. Fortunately, studies of widely distributed and relatively common species can provide, and have provided (e.g. UK National Bat Habitat Survey; Racey, 1998), valuable information that can be built into broad conservation and management plans.

In light of these issues, the importance of abundant bats should be continually emphasized. In our race to conserve rare and endangered species, we must also conserve the abundance of species such as *E. fuscus*. Their ecosystem role may vastly exceed the role of inherently rare or currently endangered species. In addition, widespread, abundant bats such as *E. fuscus* provide a wealth of research opportunities from which we may be able to draw some general conclusions about bat conservation as a whole.

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acid) τ 6.5 (singlet). Ultraviolet absorption in 0.1*N* HCl showed peaks (λ_{max}) at 231 nm ($\epsilon = 10,700$); and at 284 nm ($\epsilon = 9,650$). In 0.1*N* NaOH, λ_{max} was 269 nm ($\epsilon = 10,150$) and at 309 nm ($\epsilon = 12,800$).
12. The data for 1-methylhydantoin-5-oxime are as follows. Calculated (percent) for $C_4H_5N_3O_3$: C, 33.57; H, 3.50; N, 29.37. Values (percent) found were C, 33.30; H, 3.54; N, 29.36. The mass spectrum [*m/e* (relative intensity)] indicated 143 [parent mass ion (23)], 127 (11); 126 (50); 83 (15); 72 6(4); 70 (44); 57 (11); 56 (47); 55 (32); 54 (23); 53 (20); 43 (27); 42 (100); 41 (12); 30 (18). The infrared data (cm^{-1}) showed 3260 (s, broad); 1780 (m); 1730 (s); 1655 (s); 1440 (s); 1240 (w); 1130 (m); 1070 (m); 1000 (s) NMR (in D_2O/NaOD) showed τ 6.55 (singlet); (in trifluoroacetic acid) τ 6.9 (singlet). Ultraviolet absorption in 0.1*N* HCl showed λ_{max} at 225 nm ($\epsilon = 6300$); 283 nm ($\epsilon = 5750$). In 0.1*N* NaOH, λ_{max} was 259 nm ($\epsilon = 9000$); 311 nm ($\epsilon = 8850$).
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Isolation and Characterization of Larvicidal Principle of Garlic

Abstract. *The larvicidal principles of garlic,* Allium sativum *L., have been isolated and identified as diallyl disulfide and diallyl trisulfide. Both natural and synthetic samples of these larvicides are fatal at 5 parts per million to* Culex pipiens quinquefasciatus *Say.*

Active search for effective plant agents that will destroy mosquitoes has been prompted by the controversy concerning the general harmfulness of DDT and by the development by insect pests of resistance to various other chemical insecticides. At one time DDT was considered a panacea for eliminating the mosquito problem. Although medicinal and antibacterial properties of garlic (*Allium sativum* L., N.O. Liliaceae) have been extensively studied (*1*), only recently has the larvicidal property of its oil, for at least four species of mosquitoes in *Culex* and *Aedes* genera (*2*), been demonstrated. Greenstock (*3*) has shown that garlic oil could destroy aphids, cabbage-white butterfly caterpillars, and Colorado beetle larvae. We now report the isolation, characterization, and testing of the active principle in garlic responsible for the mosquito control. We used *Culex pipens quinquefasciatus* Say (the same as *C.p. fatigans* Wiedemann) as test organism.

The medicinal properties of garlic and related plants have prompted study of their chemical composition (*4*). Several *S*-substituted cysteines and cysteine sulfoxides, partly in the free form and partly as γ-glutamyl peptides have been isolated from various species of *Allium*. However, it has been proved that the physiologically active compounds are formed through the enzymatic reactions and spontaneous decomposition of parent compounds. The alkyl sulfides, cysteine sulfoxides, and thiols that have been reported to be present are produced by the degradations of the precursors (*1*).

In our work the crude garlic oil was obtained by steam distillation of homogenized garlic cloves. The oil was purified on a silica gel column and was eluted with solvents of increasing polarity. The fractions obtained from the column were tested for larvicidal activity as described (*2*). The fractions eluting with light petroleum had pronounced larvicidal activity. The active fraction contained sulfur. The infrared spectrum (1640, 990, and 910 cm^{-1}), nuclear magnetic resonance (NMR) spectrum (3.5 δ, 2H; 5.25 δ, 2H; 5.98 δ, 1H), and mass (*m/e*, 41) spectrum show the presence of allyl ($CH_2{=}CH{-}CH_2{-}$) group. Color reactions and absence of lowfield signal in the NMR spectrum indicated the absence a thiol group. Since there are no sulfur-oxygen absorptions (*5*) in the infrared spectrum, sulfur should be present as sulfide linkage only. Gas-liquid chromatography (GLC) (*6*) indicated the presence of several components. Two major components could be separated by preparative GLC and were subjected to mass spectroscopy. The more volatile component was identified as diallyl disulfide (*m/e*, 146) while the other fraction corresponded to diallyl trisulfide (*m/e*, 178). A trace amount of dialyl tetrasulfide (*m/e*, 210) was also indicated. The above conclusions were confirmed by comparison with synthetic preparations. Diallyl disulfide was prepared as was described by Carson and Wong (*7*). A mixture of diallyl disulfide and diallyl trisulfide was obtained by the interaction of sodium polysulfides and allyl bromide. Diallyl trisulfide could be separated from the mixture by preparative GLC (*6*). The presence of diallyl disulfide and diallyl trisulfide in the natural sample was confirmed by the infrared and mass spectra and GLC comparisons with the synthetic samples.

The larvicidal action of the natural samples has been compared to several synthetic samples as shown in Table 1. The relative effectiveness of diallyl disulfide and diallyl trisulfide alone or in mixture even at a concentration of 5 ppm, as against the ineffectiveness of the related compounds diallyl sulfide and dipropyl disulfide and dipropyl trisulfide at 200 ppm is noteworthy. We have also observed that antagonistic properties of diallyl di- and trisulfides against several pests of economic and medical importance such as potato tuber moth, red cotton bug, red palm weevil, houseflies, and mosquitoes. The nontoxic nature of garlic to higher animals has been established on the basis that it has been used for edible pur-

Table 1. Toxicity tests of active fraction of garlic oil and synthetic samples to late third-instar larvae of laboratory-reared *Culex pipiens quinquefasciatus* Say.

Compounds used	Mean percentage of mortality at indicated treatment concentrations (ppm)*							
	1	3	5	7	10	50	100	200
Natural sample†	3	64	100	100	100			
Synthetic mixture of diallyl di- and trisulfides	4	76	100	100	100			
Diallyl disulfide	4	70	100	100	100			
Diallyl trisulfide	0	49	92	100	100			
Diallyl sulfide	0	0	0	0	0	0	0	16
Dipropyl disulfide and dipropyl trisulfide	0	0	0	0	0	0	0	0

* Each mean based on five replications; 50 larvae per replicate; mortality scored after 24 hours.
† The ratio of diallyl di- and trisulfide varies with the variety of garlic used.

poses for a long time. This, together with the wide range of activity of the oil, suggests that garlic oil or its active principle, whether natural or synthetic, could be used as pesticides.

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8. We express our appreciation to A. T. Sipahimalani, K. Annaji Rao, and Dr. C. Bandyopadhyay for GLC analysis and to Dr. K. N. Rao and K. N. Bhide for mass spectra.

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Hypertension of Renal Origin: Evidence for Two Different Mechanisms

Abstract. *Antibody to angiotensin II, or a specific peptide competitive inhibitor of angiotensin II, was used to investigate the role of the renin-angiotensin system in two types of renal hypertension in rats. The data indicate that angiotensin II is in fact critically involved in the pathogenesis of the form of renal hypertension in which one renal artery is clamped and the contralateral kidney is left in place, but that it probably plays no significant role in the maintenance of experimental renal hypertension in which the opposite kidney has been removed.*

Goldblatt and co-workers first induced chronic arterial hypertension in dogs by partially occluding one renal artery and removing the opposite kidney (*1*). Subsequently, increased renin secretion by such clipped kidneys was demonstrated (*2*). Renin acts enzymatically to release angiotensin I from a plasma globulin (*3*). Angiotensin I is then converted by other enzymes to the active octapeptide angiotensin II, the most potent pressor substance known. Accordingly, it has seemed reasonable to assume that increased plasma angiotensin II is the cause of experimental renal hypertension and its various naturally occurring counterparts in man. However, numerous studies have failed to establish this assumption as fact, and both plasma renin and angiotensin II levels are often normal in chronic renal hypertension of various species, including man (*4*). Furthermore, in other studies in which antibodies to renin or angiotensin were either administered or induced it very often has not been possible to correct this type of hypertension (*5, 6*). Therefore, the etiologic role of angiotensin II in causing or maintaining renal hypertension is open to question.

In our experiments reported here, angiotensin II antibodies were administered intravenously to rats with chronic renal hypertension, and the effects were compared with the administration of a new highly specific synthetic peptide competitive inhibitor of angiotensin II. This compound, [sarcosine[1]–Ala[8]]angiotensin II, completely blocks the pressor action of exogenous angiotensin II in rats and dogs when given in approximately equimolar amounts (*7*). The compound itself has no pressor or depressor activity when given intravenously. Its biological half-life is approximately 12 minutes. The use of a similar peptide inhibitor of angiotensin II has been described (*8*).

Two types of renal hypertension were studied. In the first type a silver clip was placed on the left renal artery and the other kidney was left untouched (this is referred to as two-kidney Goldblatt hypertension). In the second, a silver clip was placed on the left renal artery, and the contralateral kidney was removed (one-kidney Goldblatt hypertension). The two groups of hypertensive animals, together with an additional control group, were maintained for 6 weeks on Purina rat chow (0.42 percent sodium content) and allowed free access to water. All animals weighed 350 to 450 g. A mean blood pressure of 121.3 ± 6.6 mm-Hg (mean ± standard error) was found in normal rats. The mean blood pressure of two-kidney hypertensive animals was 195.6 ± 10.8 mm-Hg. The difference in blood pressures between the two hypertensive groups was not statistically significant.

Antibodies to angiotensin II were prepared in rabbits (*9*). The apparent affinity constant of the antibody was calculated to be 3×10^{11} liter/mole.

The animals were anesthetized with intraperitoneal pentobarbital (5 mg/100 g). Both jugular veins were cannulated (PE-10 catheter) for injection or infusion, and the blood pressure was continuously monitored with a strain gauge through a carotid artery catheter (PE-50). During an initial control period standard doses of angiotensin II (50 ng) and of norepinephrine (100 ng) were injected through the cannula; pooled rabbit serum was then injected as a control, and the animals were challenged with angiotensin II and norepinephrine standards. Then, either undiluted serum containing angiotensin II antibody (0.3 or 0.6 ml) was injected as a single dose or, alternatively, the angiotensin II inhibitor was infused at a rate of 4 μg/min for 1 hour. After the antibody injection or during infusion of the inhibitor, the pressor effects of the standard amounts of exogenous angiotensin II and norepinephrine were checked periodically.

The blood pressure response to exogenous angiotensin II in normal rats ($n = 10$) was blocked by as little as 0.3 ml of antibody. The amount of antibody also induced an immediate fall in blood pressure of 47.5 ± 2.5 mm-Hg, which was sustained for about 5 minutes, before it gradually returned to baseline levels (in the next 10 minutes). However, the pressor effects of exogenous angiotensin II remained completely blocked for up to 3 hours. Blood pressure response to exogenous norepinephrine was never affected by the administration of the antibody. Administration of the pooled rabbit serum had no effect on the blood pressure or the pressor activity of angiotensin II or norepinephrine.

Twice as much antibody (0.6 ml) was required to abolish the pressor effect of exogenous angiotensin II in the two-kidney hypertensive rats ($n = 6$) and in the one-kidney rats ($n = 6$). However, in the two-kidney animals antibody administration induced a fall in mean blood pressure of 41.0 ± 4.0 mm-Hg ($P < .001$), whereas in the one-kidney rats the blood pressure fell by only 10.0 ± 6.4 mm-Hg, and this change was not significant ($P > .1$) (Fig. 1). The reduction in blood pressure lasted for an average of 16 min-

INSECTICIDES RECOMMENDED FOR MOSQUITO CONTROL IN NEW JERSEY IN 2009

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Introduction
Conversion factors and abbreviations
Larvicidal applications
Specifications, formulations and dilution for larvicides
Pupicidal applications
Adulticidal applications
Insecticides under observation for use in New Jersey
Precautions and Safety
New Jersey Poison Information and Education
Common names and proprietary examples
APPENDIX: Best Management Practices for Mosquito Control in New Jersey

New Jersey Agricultural Experiment Station, Publication No. P-08001-01-08.

INTRODUCTION

The New Jersey Agricultural Experiment Station annually reviews the selection of insecticides to be used by the county mosquito control commissions and agencies responsible for reducing the populations of nuisance and vector species of mosquitoes in New Jersey. This is done by faculty in the Department of Entomology who have experience in mosquito research and control, including factors of safety, economy and efficiency under New Jersey conditions. These recommendations are produced as a service to the residents of New Jersey and are for use in New Jersey only. The New Jersey Agricultural Experiment Station takes no responsibility for their use elsewhere. Selection of insecticides depends on environmental considerations and on continued information exchange between the State and Federal authorities and the professional organizations in mosquito research and control in New Jersey. Professional mosquito control in New Jersey relies on the surveillance of mosquito sources and problems and the proper consideration of options for control, such as water management, biological control, and insecticides. This integrated and comprehensive approach to the control of mosquitoes utilizes all available control strategies to reduce the

occurrence of mosquitoes as pests to tolerable levels while maintaining a quality environment.

When mosquito problems necessitate the use of insecticides, it is generally best to employ larvicides and pupicides, as they are directed efficiently at the most concentrated developmental stages of the mosquito population and reduce the need for large-scale and expensive adulticiding. Only the public county and state commissions or agencies charged with the responsibility for mosquito control may perform larviciding and pupiciding. If weather or environmental concerns prevent such efforts, adulticides can be used shortly after emergence when adult mosquitoes are still concentrated in their source area and before they have dispersed. The purpose of mosquito control is to reduce nuisance and disease potential, not to eradicate mosquitoes. In an integrated approach to mosquito control, adulticiding may be necessary for dispersed or migrating adult mosquitoes. Special attention should be given to the level of mosquito activity and the prevailing environmental conditions in order to insure maximum efficiency of the application.

All applications of synthetic or biological insecticides for larviciding, pupiciding, or adulticiding purposes must be consistent with and comply with the principles of integrated pest management (IPM) as described in the APPENDIX, entitled “Best Management Practices for Mosquito Control in New Jersey”. This text (revised in 2008) is an excerpt of all clauses pertaining to mosquito control in New Jersey from “The Environmental Protection Agency’s Pesticide Environmental Stewardship Program“ and contains detailed and comprehensive (http://www-rci.rutgers.edu/~insects/psd.htm), information on integrated practices for mosquito control in New Jersey.

A county commission or agency wanting to test an insecticide not included in these recommendations can request, in writing, support for such testing from the New Jersey Agricultural Experiment Station (NJAES) (write to or e-mail Dr L. B. Brattsten, Rutgers University, Department of Entomology, Blake Hall, 93 Lipman Drive, New Brunswick, NJ 08901; brattsten@aesop.rutgers.edu). Please include in your letter details of locations and target species. All such applications of insecticides should follow the manufacturer’s recommendations. For the benefit of mosquito control in New Jersey, information gathered on control efficacy for the target species and any effects on non-target species should be shared with members of the Associated Executives of Mosquito Control Work in New Jersey and the NJAES.

Just as bacteria evolve resistance to antibiotics, mosquitoes evolve resistance to insecticides used for their control. Selection for resistance can result from the repeated use of the same insecticide exclusively and from slow-release formulations of insecticides. To avoid or delay resistance, a variety of different insecticides and other control methods must be used in rotation. Reliance on a single insecticide frequently or over large contiguous areas is likely to produce resistance to that control agent. The three major larvicides recommended for use in New Jersey, temephos, S-methoprene, and BTI are ideal for use in rotations as each has low environmental stability, a separate molecular mode of action, and significant differences in detoxification mechanisms. For adulticiding, malathion is the least likely to trigger evolution of resistance and synergized pyrethroids the most likely.

For any application of an insecticide by a mosquito control professional, including any pesticide application in public places, the applicator or the direct supervisor must be certified

and licensed as a “Commercial Pesticide Applicator” by the NJ DEP PCP Bureau of Pesticide Operations (N.J.A.C. 7:30-6). The correct license category is Category 8B - Mosquito Control. The Category 8C – Campground Pest Control license may be appropriate under certain circumstances. Pilots applying insecticides to control mosquitoes must have Category 11 - Aerial Pest Control in addition to 8B - Mosquito Control. The same holds true for research and demonstration applications, Category 10 - Demonstration and Research Pest Control and 8B - Mosquito Control are required. Training manuals may be obtained from your local county cooperative extension office. Certified and licensed applicators may have employees applying insecticides using non-aerial equipment under their direct supervision provided that these employees are registered as “Commercial Pesticide Operators” (N.J.A.C. 7:30-5). Contact the New Jersey Department of Environmental Protection, Pesticide Control Program (www.pcpnj.org) in Trenton for additional information regarding pesticide applicator permit details, 1 609 984 6614.

Common names of insecticides (along with Trade Names®) are used in the interest of increasing the understanding of the materials used. The trade or brand names given herein are supplied with the understanding that no discrimination is intended (against similar products not mentioned) and no endorsement by the New Jersey Agricultural Experiment Station is implied.

CONVERSION FACTORS AND ABBREVIATIONS

To aid in the use of the metric system, the rates and dilutions in these recommendations are given in the metric system units with the United States equivalent in parenthesis. Below is a list of the conversion factors and abbreviations used in these recommendations:

1 kilogram, kg = 2.2 pounds, lbs	1 fl ounce, oz = 29.6 mL
1 gram, g = 0.002 lbs	1 lb/acre = 1.12 kg/ha
1 hectare, ha = 2.47 acres	1 pint/acre = 1.17 L/ha
1 liter, L = 1.056 quarts	1 quart/acre = 2.34 L/ha
1 milliliter, mL = 0.001 L	1 fl oz/acre = 73.2 mL/ha

active ingredient (AI); aqueous suspension (AS); emulsifiable concentrate (EC); extended release (XR); kilometer per hour (kph); international toxic units (ITU)

LARVICIDAL APPLICATIONS

The following insecticides are recommended for the control of larvae of nuisance and vector species in various larval habitats. The insecticides are listed alphabetically, not in order of expected efficacy.

A. Catch basins

(1) Temephos (Abate®) emulsion or 5% extruded pellets according to product label.
(2) S-methoprene (Altosid®, Aquaprene®) pellets and standard briquets according to product label.

(3) Monomolecular films (Agnique®MMF, Golden Bear Oil, *i.e*. Mosquito Larvicide GB-1111®) as a larvicide/pupicide, according to product label.
(4) *Bacillus thuringiensis israelensis* serotype H-14 (BTI) briquets or other formulations, including Aquabac®, Teknar®, Vectobac®, Bactimos® (see B3).
(5) *Bacillus sphaericus* (BSP) (see C4), including Vectolex® WSP (7.5% BSP) water-soluble pouch for use in catch basins.

B. Fresh flood water areas, woodland pools

(1) Temephos (Abate®) emulsion, granules, 5% extruded pellets according to product label.
(2) S-methoprene (Altosid®, Aquaprene®) liquids, pellets, or briquets (standard) according to label.
(3) BTI according to manufacturer's directions. BTI must be ingested to be toxic to mosquito larvae. Larval toxicity depends on the species, its feeding activity and various environmental factors. BTI formulations (*e.g*., flowables, briquets, granulars, and pellets) may vary in their potency and the means used to express such potency (*e.g*., ITU/g). Attention should be given to this aspect in the purchase and use of BTI products. Where such formulations are meant to be suspended in water for application, agitation must be provided to insure uniform application.

C. Polluted and/or impounded waters

(1) Temephos (Abate®) emulsion, 1 or 2% granules, 5% extruded pellets according to product label. See also E3.
(2) S-methoprene (Altosid®, Aquaprene®) liquids, pellets, briquets (standard) according to label.
(3) Monomolecular films (Agnique®MMF, Golden Bear Oil, *i.e*., Mosquito Larvicide GB-1111®) as a larvicide/pupicide, according to product label.
(4) BSP recommended for the control of *Culex* larvae: use according to manufacturer's directions. BSP may also be an effective larvicide for non-*Culex* species.

D. Prehatch for woodland pools with a record of annual early larval activities

(1) S-methoprene (Altosid®, Aquaprene®) pellets briquets (standard) according to product label.
(2) BTI briquets, according to product label.

E. Salt marsh

(1) The use of larvicides on the open tidal marsh should be in conjunction with a plan involving water management for long-term reduction of the mosquito problem and of insecticide use. Heavy rains or exceptionally high tides may make it necessary to larvicide in defined areas.
(2) Temephos (Abate®) 2% or 5% granules, emulsion, in sufficient water to accomplish efficient distribution at rates according to product label.
(4) S-methoprene (Altosid®, Aquaprene®) (see B2).
(5) BTI Neither flowable nor granular formulations of BTI used on salt marshes have been consistently efficient in the recent past (see B3).

F. Fresh water marsh

(1) Temephos (Abate®) (see B1).
(2) S-methoprene (Altosid®, Aquaprene®) (see B2); liquids, pellets, briquets (standard) according to product label.
(3) BTI (see B3).

SPECIFICATIONS, FORMULATIONS and DILUTIONS for LARVICIDES

Emulsifiable concentrates

These formulations, which are to be diluted generally with water prior to spraying, may contain a small percentage of volatile solvent.

Dilution of concentrates

Temephos (Abate®) emulsions are prepared from a 4E concentrate 0.48 kg/L (4 lbs/ gallon). For most larviciding, dilute 36.6 mL in 93.5 L water/ha (0.5 fl oz in 10 gallons water/acre). For waters high in organic matter content, the concentration may be increased 2-3 fold.

Granular larvicides

All granular formulations should be formulated to insure efficient release of the insecticide in water. Temephos (Abate®) granules may employ carriers such as sand, celatom, Plaster of Paris, or Biodac® (a cellulose product); inclusion of an oil solvent does not appear necessary. S-methoprene (Altosid®, Aquaprene®) carriers include Plaster of Paris and Biodac®. No highly volatile solvents should be used in granular formulations. For celatom carrier, the optimum particle size for aircraft application is 24/48, with no more than 15% above 48 mesh, to minimize drift. For ground application, 30/60 to 60/80, depending on equipment, is optimum; more than 10% (by weight) in particles outside these specified size ranges is considered unsatisfactory.

PUPICIDAL APPLICATIONS

The pupal stage is the briefest stage in the development of the mosquito. Although pupae are unaffected by organophosphates, pyrethroids, and other nerve poison type insecticides, BTI, or S-methoprene, there are some very effective pupicides. These agents will also control fourth instar larvae, which may be present when it is necessary to pupicide. As with larviciding, timely efforts to control concentrated populations of pupae can be of value in preventing the emergence of adult mosquitoes and reducing the need to adulticide. The following insecticides are recommended for the control of pupae of nuisance and vector species.

- Monomolecular films (Agnique®MMF, Golden Bear Oil 1111, Bonide Mosquito Larvicide) according to product labels.

ADULTICIDAL APPLICATIONS

While the control of mosquitoes is generally most efficiently accomplished in the immature stages, conditions may sometimes necessitate the use of adulticides. If agencies other than county mosquito control commissions or agencies responsible for mosquito control wish to adulticide, they should contact the NJ Pesticide Control Program (609-984-6666) concerning regulations. Community or area-wide notification of adulticiding is required according to NJAC 7:30-9.10 (www.pcpnj.org).

Particular attention should be given to temperature, as it may affect droplet behavior, and the toxicity of the insecticide to the target mosquito. Of the types of adulticide recommended, the organophosphate malathion has a positive temperature coefficient, *i.e.,* more toxic at higher temperatures; resmethrin and other pyrethroids have a negative temperature coefficient, *i.e.*, they are more toxic at lower temperatures. Ambient temperature, therefore, can influence the selection of the insecticide.

The New Jersey Agricultural Experiment Station recommends the following adulticide measures. Synergized pyrethroid formulations (Scourge®, Anvil®, Duet®) should be used as rarely as possible and not over large contiguous areas to avoid or delay insecticide resistance evolution in local populations. Synergized pyrethroid formulations are most appropriately used only for barrier treatments.

A. Adulticiding with ground equipment

1. Thermal aerosols, fogging

Insecticide fogging can be an effective method in mosquito control. It is not meant to be used routinely but only when populations of adult mosquitoes reach public health or nuisance levels. These levels are highly variable and depend on the mosquito species involved as well as local environmental conditions. The final decision to fog should rest with the mosquito control professionals in each county. Trained personnel at these institutions are expertly knowledgeable about local mosquito populations and conditions. When fogging is deemed necessary, the following physical conditions, mostly encountered in the early evening and morning hours, should exist:

a. Air temperature: 15°C or higher (60°F)
b. Light intensity: below 20 foot candles, with light meter
c. Wind velocity: 5-8 kph (3 to 5 mph)
d. Stable thermal conditions to allow the fog to travel at ground level.

The only material recommended for fogging is malathion. The 95% malathion concentrate should be diluted as follows for fogging: 15 L (4 gal) concentrate is added to sufficient solvent to make 379 L (100 gal) final volume (0.4%). The diluted material is applied at a flow rate of 151 L/h (40 gal/h) with a vehicle speed of 8 kph (5 mph). An experienced and knowledgeable operator and a properly equipped vehicle and fogger are absolutely essential.

Per label, attention should be given to the flash point of the solvent used as measured by the “closed cup” method.

2. Sprays by mist blowers and hydraulic sprayers

While mainly intended for use with residual insecticides, this equipment can be employed to apply dilute emulsions of the non-persistent insecticide malathion to foliar surfaces for short-term residual mosquito control. The materials should be diluted and applied according to label recommendations for such equipment.

3. ULV (ultra low volume) spray applied by ground equipment

The technique of ULV has the advantage over fogging of being less dense and, therefore, less hazardous in urban traffic. Physical conditions as stated for fogging are generally desirable, and application should coincide with times of maximum adult mosquito activity in order to achieve maximum efficiency.

The technique of ULV employs more concentrated insecticides and the equipment for their application must be properly calibrated and serviced. Ground ULV applications do not always penetrate dense foliage as well as do fogging applications. Application of any ground ULV material should be performed under conditions also known to be best for efficient fogging operations (A1 above); wind speeds up to 16 kph (10 mph) are acceptable. Application should be made after sunset or before sunrise at temperatures of 15 to 28°C (60 to 82°F).

(a) Malathion (Fyfanon®, Atrapa®, 96-98%) at the flow rate of 90 mL/min at 16 kph or 45 mL/min at 8 kph (3 fl oz/min at 10 mph or 1.5 fl oz/min at 5 mph). With a constant volume flow meter and depending on conditions, *e.g.*, acreage to be treated and period of mosquito activity, application may be made at 20 mph. According to the labeling of these products, their application by ground ULV is restricted to professional mosquito control personnel who have the experience, knowledge and equipment necessary to follow the technical instructions for their use.

(b) Pyrethroid/piperonyl butoxide mixtures such as Scourge® (resmethrin/piperonyl butoxide in a 1:3 ratio), Anvil® (d-phenothrin/piperonyl butoxide 2+2) or Duet® (d-phenothrin/ prallethrin/ piperonyl butoxide – 5/1/5). Scourge® (4 +12) is available in a formulation for use without further dilution. Use these products according to instructions on the label and as seldom as possible.

B. Adulticiding by aircraft

Application from aircraft may only be performed according to Federal Aviation Regulations, by the county mosquito control commissions, equivalent county units, or the State Mosquito Control Commission using materials specifically labeled for application by aircraft (N.J.A.C. 7:30-6.3). To insure that the droplets descend from the aircraft to the areas of mosquito activity, these applications should be made close to sunset or thereafter, or early morning, when a deep temperature inversion occurs. For further discussion of this aspect see A. V. Havens, *Proc. N. J. Mosq. Ext. Assoc.*, **60:**59-63 (1973).

1. LV (low volume) spraying

(a) 148 mL of 96-98% malathion per 2.3 L of No. 2 fuel oil/ha (2 fl oz 95% malathion in 1 quart of solvent/acre) (EPA SLN No. NJ-950003).

2. ULV (ultra low volume) spraying

(a) 220 mL/ha (3 fl oz /acre) of 96-98% malathion as applied by fixed wing aircraft or helicopter equipped with a rotary atomizer (*e.g.*, a Beecomist nozzle) according to insecticide manufacturer's specifications with the additional stipulation that wind velocity be no greater than 8-16 kph (5-10 mph). To insure that the equipment performs correctly and produces proper droplet sizes, the equipment should be periodically calibrated and examined closely. Systems should include elements for positive shutoff of delivery. Spray droplet size should be determined periodically. To prevent malfunction of the system, the malathion should be filtered just prior to use by a method similar to that described by H. R. Rupp, *Mosquito News*, **33:**463-464 (1973).

(b) Resmethrin/Piperonyl butoxide in a ratio of 1:3 by weight, such as Scourge® at rates according to the label.

INSECTICIDE FORMULATIONS UNDER OBSERVATION FOR USE IN NEW JERSEY

Several other released materials could be useful for mosquito control in New Jersey. They have not yet been fully investigated for suitability. We recommend that exploratory applications be performed with the following:

- Anvil® (d-phenothrin 10% + PBO 10%) for ground or aerial adulticiding
- Aqua-Reslin® (permethrin 20% + PBO 20%) for barrier treatments
- Duet® (d-phenothrin/ prallethrin/ piperonyl butoxide – 5%/ 1%/ 5%)

These applications should be relatively small-scale and employ a variety of measurements of effectiveness, *e.g.*, caged mosquitoes, light trap counts, or landing counts. It would also be of interest to make qualitative observations of possible effects on non-target organisms. NJAES relies on results from experimental applications to use for decisions of the inclusion of these materials in the recommended insecticides.

PRECAUTIONS AND SAFETY

Most insecticides are not only toxic to mosquitoes but can also be toxic to humans and other forms of life in the environment. It is necessary for all persons responsible for the use of insecticides to recognize this and take precautions to insure that these chemicals not only do not cause human illness or death but also absolutely minimally contaminate the environment. Further information is available from the National Pesticide Information Center (1 800 858 7378 or at npic.orst.edu). Public notice about planned spray operations must be issued according to NJAC 7:30-9.10 (www.pcpnj.org).

Manufacturers are required by law to list on the insecticide label those precautions to be followed to reduce hazards. Such precautions include not only appropriate concentrations to be used but also protective clothing for applicators, antidotes for poisoning, and conditions of storage.

Precautions should also be taken at other times. Insecticides should be stored in a manner inaccessible to people who are not knowledgeable of their toxicity and hazards. Storage should be in an area set aside solely for that purpose, and the area should be well ventilated to prevent overheating and subsequent noxious fumes of solvents or insecticides. When empty, insecticide containers should be triple rinsed, punctured and disposed of according to the product label, or returned to the supplier. Containers should not be burned because of air pollution by smoke and residual insecticide in the containers. Unused insecticides should not be discarded in drainage systems but should be turned over to authorized agencies for appropriate disposal. Regulations for storage can be found at NJAC 7:30-9.5 and 9.6, and general regulations on disposal are at 7:30-9.7. More specific requirements and guidance for the disposal of waste are available from NJ DEP PCP at 1 609 530 4070 or www.pcpnj.org or from NJDEP at 1 877 927 6337.

NEW JERSEY POISON INFORMATION AND EDUCATION

During the mixing and application of insecticides, all precautions listed on the insecticide labels should be followed. We recommend establishing background acetylcholinesterase activity levels by the appropriate test in all personnel working with organophosphate insecticides, during periods of extended use, and periodically thereafter. For information and aid regarding acute insecticide poisoning, call either the NJ Poison Information and Education System, 1 800 222 1222; http://www.njpies.org or the National Pesticide Information Center, 1 800 858 7378; http://npic.orst.edu/ . Please consult available web sites for extensive information on safety and other aspects on insecticides.

NEW JERSEY POISON INFORMATION
AND EDUCATION SYSTEM
1 800 222 1222
or
www. njpies.org

COMMON NAMES AND PROPRIETARY EXAMPLES

Common name	Proprietary examples®
Bacillus sphaericus (BSP)	Vectolex
BTI	Bactimos, Teknar, Vectobac, Aquabac
Ethoxylate surfactant	Agnique MMF
Malathion	Fyfanon, Atrapa
S-Methoprene	Altosid, Aquaprene
Permethrin/piperonyl butoxide	Aqua-Reslin
Petroleum derivative	Mosquito Larvicide GB 1111
d-Phenothrin/piperonyl butoxide	Anvil, Sumithrin
Resmethrin/piperonyl butoxide	SBP-1382, Scourge
d-phenothrin/ prallethrin/ piperonyl butoxide	Duet
Temephos	Abate

APPENDIX

"Best Management Practices for Mosquito Control in New Jersey"*

OVERVIEW OF MOSQUITO CONTROL IN NEW JERSEY

New Jersey has a diverse ecology that provides habitat for more than 60 species of mosquitoes. New Jersey also has more human residents per square mile than any other state. At the turn of the 20th century, New Jersey functioned as the center for mosquito research and the early (http://www-rci.rutgers.edu/~insects/wiab.htm) workers developed many of the basic concepts used in mosquito control today. Their successes led to the creation of organized, multidisciplinary mosquito control as a proper function of government. Information sharing among researchers and control workers was recognized as an important component of responsible mosquito management and was formalized in the early 1900's.

The philosophy of mosquito control in New Jersey is to target mosquitoes and/or their habitat as specifically as possible in a financially efficient manner. Minimizing insecticide impact on non-target organisms has always been vital to public acceptance and was incorporated into the goals of the mosquito control community. The need to be specific in

the selection and application of insecticides is fundamental to the methods we use in New Jersey.

The sanitation and habitat modification procedures developed or enhanced by the early mosquito control workers in New Jersey form the foundation for today's source reduction activities. The water management techniques pioneered by New Jersey's early workers have been honed into the most efficient long term methods available today to reduce mosquito production. Enhancement of natural predators was deemed important in the early days of mosquito control and is now an accepted component of New Jersey's program that is funded and coordinated by our state agencies.

Surveillance is one of the best tools we have for focusing mosquito control on specific pest and vector species. Sampling and identification allow problem species to be recognized and targeted for control. Early mosquito control workers in New Jersey benefited greatly from the landmark investigations of John B. Smith (http://www-rci.rutgers.edu/~insects/early.htm), a legendary taxonomist and founder of organized mosquito control. Surveillance programs to monitor disease organisms and their mosquito vectors were not available to early workers but are fundamental to New Jersey's programs today. In New Jersey, the need for control, type of management employed and alternatives to insecticides are all weighed against the surveillance data we collect.

The history of mosquito control in New Jersey shows long standing environmental awareness and the ability to select insecticides, only when necessary, from the broad array of techniques we have at our disposal.

NEW JERSEY'S CONCEPT OF A RESPONSIBLE MOSQUITO CONTROL PROGRAM

Mosquito control in the state of New Jersey is mandated by law under Title 26, Chapters 3 & 9 of the NJ Health Statutes.

Title 26 assigns the control of pest and vector species to county mosquito control commissions which function as autonomous units of county government. Activities and expenditure of funds are overseen by a body of commissioners appointed by the board of chosen freeholders in each county. Tax levies provide the operational budget on a county-by-county basis. Autonomous mosquito commissions have the powers of a local board of health regarding mosquitoes including the right of entry onto public and private properties. They have the power to make a declaration regarding mosquito nuisance and can issue an abatement notice whenever necessary. Seven New Jersey counties currently maintain autonomous commissions and 14 counties have mosquito control responsibilities assigned to other agencies of county government.

The laws enacted by Title 26 mandate the Director of the NJ Agricultural Experiment Station (NJAES) at Rutgers University to function in an advisory capacity to all mosquito control agencies in the state. Specific duties of the Director include: 1) annual review of mosquito commission plans & estimates, 2) conducting surveys for county agencies upon request, 3) investigating the life histories of individual species, 4) recommending methods for control, and 5) conducting extension related activities that educate the public and advocate responsible mosquito control. A primary objective of NJAES involvement in Title 26 is to

maintain professionalism within the mosquito control community in New Jersey that is consistent with current environmental concerns.

Title 26 also provides for a State Mosquito Control Commission (SMCC) that functions in an advisory capacity to the Governor. Composition of the SMCC includes 6 public members appointed by the Governor and representatives from the New Jersey Department of Environmental Protection (NJDEP), the NJ Department of Health and Senior Services (NJDHSS), the NJ Department of Agriculture (NJDA) and the NJAES. The SMCC is mandated to carry on a continuous study of mosquito control operations in the state, recommend the amounts of money deemed necessary for mosquito control purposes and allocate state aid to counties from an annual appropriation. The Office of Mosquito Control Coordination (OMCC), within the NJDEP, administers SMCC funding and expedites operational programs advocated by that body. Representation of the above mentioned departments of state on the SMCC board fosters a network of communication that recognizes the interdepartmental nature of mosquito control problems and activities in the state.

The operational aspects of mosquito control in New Jersey are conducted by the autonomous mosquito control commissions described above, mosquito control agencies within other county departments as well as federal, municipal and private mosquito control programs. Regardless of the agency, the NJMCA advocates the following as necessary components of responsible programs.

A. SURVEILLANCE. NJ believes that mosquito control begins with a surveillance program that targets pest and vector species and justifies the need for control. We believe that species-specific records should be kept on the composition of mosquito populations prior to enacting control of any kind. We also advocate records on the composition of mosquito populations after management to determine the effectiveness of control operations. The New Jersey light trap was designed as a surveillance tool more than 50 years ago for that purpose. Most mosquito control agencies use light traps in their programs but have additional tools that provide data to guide their activities. The following list of surveillance methods is available for use by mosquito control agencies in New Jersey.

1. Larval Surveillance. Larval surveillance involves sampling a wide range of aquatic habitats for the presence of pest species during their developmental stages. Most counties have a team of inspectors to collect larval specimens on a regular basis. A mosquito identification specialist normally has the task of identifying the larvae to species. Properly trained mosquito identification specialists can separate mosquito species that cause nuisance and disease from those that are non-pests or beneficial species. Responsible control programs target pest populations for control and avoid managing habitat that supports benign species.

2. Adult Surveillance. Adult surveillance measures mosquito populations that have successfully developed and emerged from aquatic habitats. The New Jersey light trap has been the standard for collecting adult mosquitoes and most county agencies operate light traps from early May through October. Portable traps baited with carbon dioxide are

useful in areas where electricity is not available. Not all mosquito species are attracted to light and other forms of adult surveillance are frequently employed, *e.g*., gravid traps. In coastal areas of New Jersey, 1-minute landing rates are used to assess the comparative size of host seeking salt marsh mosquitoes during daylight hours. At inland areas, 10-minute bite counts measure annoyance after dark. Resting boxes are frequently used to measure populations of *Culiseta melanura*, a bird-feeding mosquito that functions in the amplification of eastern equine encephalitis (EEE) virus. Bird-baited traps are sometimes employed to measure *Culex* mosquitoes that amplify St. Louis encephalitis virus.

3. Virus Surveillance. The New Jersey SMCC funds a virus surveillance program that estimates the size of virus vector populations during the summer season and tests specimens for virus presence weekly. Mosquito collections are made at permanent study sites by staff from the NJAES. A wide range of assistance and support is provided by local mosquito control agencies in this effort. Specimens are tested for virus at the NJDHSS and some county laboratories by immunoflourescent antibody and polymerase chain reaction (PCR) technology. In addition, some county mosquito control agencies run sentinel chicken programs to identify areas where mosquito-borne encephalitis virus is active and test mosquitoes for virus by PCR to keep their control activities current. The status of mosquito borne encephalitis virus is disseminated to all mosquito control agencies in the State in a weekly summary throughout the encephalitis season.

B. **SOURCE REDUCTION.** Source reduction is the alteration or elimination of mosquito larval habitat. This remains the most effective and economical method of providing long-term mosquito control in New Jersey. Source reduction can include activities as simple as the removal of used tires and the cleaning of rain gutters and bird baths by individual property owners, to extensive regional water management projects conducted by mosquito control agencies on state and/or federal lands. All of these activities eliminate or substantially reduce mosquito breeding and the need for repeated applications of insecticides in the affected habitat. Source reduction activities within New Jersey can be separated into the following two general categories:

1. Sanitation. The by-products of the activities of people have been a major contributor to the creation of mosquito larval habitats. An item as small as a bottle cap or as large as the foundation of a demolished building can serve as a mosquito larval habitat. Sanitation is a major part of all IPM programs exemplified by tire removal, de-snagging waterways, catch basin cleaning, and container removal.

Mosquito control agencies in New Jersey have statutory police powers that allow for due process and summary abatement of mosquito-related public health nuisances created on both public and private property. The sanitation problems most often resolved by agency inspectors are problems of ignorance, neglect, oversight or laziness on the part of property owners. Collectively, they result in a major use of agency manpower and resources.

Educational information including videos, slide shows and fact sheets distributed at press briefings, fairs, schools and other public areas have information regarding the importance of sanitation. We must continue to emphasize the role of sanitation as an effective mosquito control modality that is a cost effective, low tech, high result method

of preventing disease potential and mosquito interference with our ability to enjoy the outdoors.

2. Water Management. Water management for mosquito control is a form of source reduction that is conducted in fresh and saltwater larval habitats.

a. *Freshwater Wetlands Management* - In 1987 the NJ State Legislature enacted into law the New Jersey Freshwater Wetlands Protection Act (NJSA 13:9b-1 *et seq*.) All ditch maintenance, stream and storm water basin cleaning, and/or restoration activities for mosquito control are now regulated by the NJDEP. *Best Management Practices for Mosquito Control and Freshwater Wetlands Management* (BMP) (NJMCA Proceedings, 2002, p.88) have been compiled through the cooperative efforts of the mosquito control community, the NJDEP and other state and federal environmental agencies. These practices are applicable to mosquito control activities in stream corridor wetlands, isolated freshwater wetlands, palustrine wooded wetlands, and storm water facilities. Using mosquito surveillance data and BMP's, New Jersey's mosquito control agencies now conduct water management activities in the state's freshwater wetlands under a number of different "statewide general permits" (*i.e*. GP-1, GP-7, GP-15) or individual permits when necessitated by the complexity of the project.

In the past, the absence of design and maintenance standards for storm water management facilities throughout New Jersey resulted in many of the facilities becoming major mosquito producers. In the late 1970's, a 4-year study of storm water facilities in New Jersey showed that due to poor design, construction and/or lack of maintenance, 67% of all basins surveyed contained mosquito larval habitat with some facilities found to be suitable habitat for up to 8 mosquito species.

In 1989, a storm water management facilities maintenance manual was produced by NJDEP. The manual is available to all developers, engineers and planning agencies statewide. This document contains specific guidelines and recommendations relative to design, construction and maintenance of storm water facilities and mosquito control ( ).

b. *Salt Marsh Water Management* - Control of the aquatic stage of the mosquitoes that are produced on New Jersey's tidal wetlands requires a complete understanding of tidal marsh ecology. Two water management techniques were developed in New Jersey to control salt marsh mosquito larval populations through the cooperative efforts of county mosquito control agencies, Rutgers University, the State Division of Fish, Game and Wildlife, the Environmental Protection Agency and the U.S. Army Corps of Engineers. These are Tidal Restoration of Salt Hay Impoundments (TRSHI) and Open Marsh Water Management (OMWM), practices that now serve as models for water management activities worldwide.

TRSHI (P. Slavin, J. Shisler,& F. Ferrigno, 1978. Current status of tidal restoration of salt hay impoundments for mosquito control in Cumberland County, New Jersey, NJMCA Proceedings, p. 214) involves the removal and/or modification of ditch plugs and other water control structures to permit daily tidal inundation to occur in salt hay impoundments. Salt hay farming was once a major industry in the Delaware Bay area of New Jersey with over 11,000 acres of salt hay impoundments located in the counties of Cape May and Cumberland. These impoundments created ideal conditions for the

production of salt marsh mosquitoes and required repeated applications of insecticides each season to control the larval populations originating in the impounded areas. The daily tidal exchange as a result of TRSHI eliminates mosquito breeding and eventually restores the area to a productive salt marsh. Over 7,500 acres of salt hay impoundments in New Jersey have been restored using TRSHI. Although TRSHI is utilized extensively to control mosquito production in salt hay impoundments, the techniques are also applicable to control mosquito breeding in other impounded marshes.

Open Marsh Water Management was developed in New Jersey in the mid-1960s through the cooperative efforts of mosquito control and wildlife agencies. OMWM (K. W. Bruder, 1980. The establishment of unified open marsh water management standards in New Jersey, NJMCA Proceedings, p. 72.) standards have been established for use by county mosquito control agencies, which address how and where the technique should be implemented. OMWM is now the major source reduction technique used by coastal mosquito control agencies in New Jersey. OMWM has been found to effectively control mosquito production on salt marshes through a combination of biological control and habitat manipulation. Three basic alterations are employed in OMWM, the construction of: 1) permanent ponds, 2) pond radials and, 3) tidal ditches. The selective excavation of the ponds, pond radials, and ditches eliminate mosquito breeding sites and provide permanent habitat for mosquito-eating killifish. In areas where OMWM is practiced, pesticide applications are substantially reduced.

C. CHEMICAL CONTROL. When source reduction and water management are not feasible or sufficient, chemicals are carefully used to control both adult and immature mosquito populations. The chemicals used by New Jersey's mosquito control agencies comply with state and federal requirements, as well as recommendations provided annually by the NJAES. All pesticide applicators and operators in New Jersey are required to be licensed by the NJDEP. Judicious chemical control activities, as part of New Jersey's IPM approach to reducing mosquito populations, use the most appropriate products available to the professionals of the mosquito control community. Chemical treatments can be directed against either the immature or adult stage of the mosquito life cycle.

1. **Larviciding.** Larviciding, the application of chemicals to kill the immature stages of mosquitoes by ground or aerial treatments, is typically more effective and target specific than treating adults. The objective is to target the immature stages at the larval habitat before populations have had a chance to disperse. New Jersey's IPM approach to mosquito control emphasizes larviciding only when source reduction is not feasible. Larvicides are applied to fewer acres than adulticides because treatments are made to relatively small areas where larvae are concentrated as opposed to larger regions where adults are present. The larvicides used for mosquito control in New Jersey include: *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* (bacterial larvicides), S-methoprene (insect growth regulator), temephos (organophosphate), and petroleum oils.

2. **Adulticiding**. Adulticiding is the use of chemicals to reduce adult mosquitoes by ground or aerial applications. Adulticiding is used when biting populations reach critical levels. In New Jersey, adulticides are commonly applied as an Ultra-Low Volume (ULV) spray in which the small amounts of active ingredient range from 0.0035 to 0.24 lb/acre.

The adulticides are dispensed through properly maintained and calibrated equipment. Adulticides used in New Jersey include pyrethroids and malathion (an organophosphate).

3. **The New Jersey State Airspray Program**. This program was established by state legislation in 1949 to assist coastal counties in the control of salt marsh mosquitoes. This ongoing program is now coordinated through OMCC within the NJDEP. Over the past decade this program has integrated a number of newer management techniques to provide for a more environmentally sound approach to pesticide applications. Emphasis is now focused on larviciding and an increased reliance on biorational pesticides. Many of the changes in the airspray program philosophy have been fostered from relationships cultivated between NJMCA members and federal and state wildlife refuge managers.

D. BIOLOGICAL CONTROL. Biological control is the manipulation of natural agents and their by-products to control pest and vector species. Biological control is advantageous because it is generally host-specific with limited non-target effects. In New Jersey, fish are the primary biological control agent used to suppress mosquito populations. Predacious fish, typically *Gambusia* species, are reared and stocked in mosquito larval habitats.

For many years, individual county mosquito control agencies raised and released their own fish. In 1990, the State of New Jersey established a statewide mosquito fish program with a specific protocol for use. With annual funding from the SMCC, the program utilizes the existing resources of the Division of Fish, Game and Wildlife's staff, hatchery and other facilities. While originally designed for *G. affinis*, the program now offers other species for mosquito control including the fathead minnow, the freshwater killifish and two species of sunfish. The use of State resources has expanded the concept of predatory fish for biological mosquito control in New Jersey.

E. EDUCATION.

1. **Continuing Education**. Continuing education is directed toward operational workers to instill or refresh knowledge related to practical mosquito control. Training is primarily in safety, applied technology and requirements for our State's regulated certification program. Examples of continuing education include: the NJMCA Pesticide Training Program, State-mandated Right to Know training for hazardous substances, the Northeast Aerial Applicator's Conference, monthly meetings of the Associated Executives of Mosquito Control Work in NJ, the annual meeting of NJMCA and meetings of other mosquito control associations in our geographic area.

2. **Public Education**. Public education is designed to teach the general public mosquito biology and to encourage residents to use simple preventive sanitation techniques. Examples include: fact sheets and brochures, classroom lectures at schools, slide shows, films and videos on mosquitoes and their control, and exhibits at fairs. NJMCA regularly interacts with civic leaders, politicians and professionals through the annual conventions of the NJ Educational Association and the NJ League of Municipalities. NJMCA produces and distributes proceedings of its annual meeting and

coordinates activities in support of the recently enacted national Mosquito Control Awareness Week. NJMCA believes that public education reduces homeowner insecticide applications and the general misuse of toxic materials. Public education encourages support for organized mosquito control rather than crisis management, which relies heavily on insecticides.

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URBAN LAND USE PREDICTS WEST NILE VIRUS EXPOSURE IN SONGBIRDS

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Abstract. Urbanization is a widespread phenomenon that is likely to influence the prevalence and impact of wildlife pathogens, with implications for wildlife management and public health policies toward zoonotic pathogens. In this study, wild songbird populations were sampled at 14 sites along an urban–rural gradient in the greater metropolitan Atlanta (Georgia, USA) area and tested for antibodies to West Nile virus (WNV). The level of urbanization among sites was quantitatively assessed using a principal component analysis of key land use characteristics. In total, 499 individual birds were tested during the spring and summer over three years (2004–2006). Antibody prevalence of WNV increased from rural to urban sites, and this trend was stronger among adult birds relative to juveniles. Furthermore, antibody prevalence among Northern Cardinals (*Cardinalis cardinalis*) was significantly higher than in other songbird species along the urban gradient. Findings reported here indicate that ecological factors associated with urbanization can influence infection patterns of this vector-borne viral disease, with likely mechanisms including changes in host species diversity and the tolerance or recovery of infected animals.

Key words: antibody presence; Cardinalis cardinalis; *host–pathogen interactions; Northern Cardinal; spatial epidemiology; urbanization; vector-borne disease; West Nile virus (WNV); wildlife disease; zoonotic pathogens.*

Introduction

Increasing urbanization, characterized by drastically altered landscapes and dense human populations, is a worldwide phenomenon. Fully two-thirds of the world's population, or 4.9 billion people, are expected to reside in cities by 2030 (United Nations Department of Economic and Social Affairs 2005). Studies exploring the influence of urban landscapes on wildlife ecology demonstrate lowered biodiversity and shifting community assemblages (McKinney 2002, Olden et al. 2006), changes in interspecific competition, individual stress and reproduction, and altered trophic interactions in these urbanized areas (Faeth et al. 2005, Partecke et al. 2006, Shochat et al. 2006).

Urbanization can also affect the dynamics of infectious diseases in wildlife, with several recent articles pointing toward potential underlying mechanisms such as altered host contact rates, changes in vector ecology, or factors that affect host susceptibility to infection (Patz et al. 2004, Bradley and Altizer 2007). For example, recent work by Farnsworth et al. (2005) showed that chronic wasting disease is significantly more prevalent in mule deer (*Odocoileus hemionus*) populations inhabiting developed areas than in those in natural areas. This is potentially due to increased contact with the infective agent, or to higher rates of intraspecific contact as a result of habitat loss. In another example, Cooper's Hawks (*Accipiter cooperii*) nesting in urban areas demonstrated more than double the nest failure rate of hawks residing in more rural environments; a likely cause was trichomoniasis, which was observed more commonly in the urban-dwelling hawks (Boal and Mannan 1999).

West Nile virus (WNV; Flaviridae; *Flavivirus*) is a vector-borne zoonotic virus maintained in avian hosts and principally transmitted by mosquito species in the *Culex* genus (Peterson et al. 2004). After the initial introduction of WNV to North America in New York City in 1999, the virus rapidly spread and reached Georgia by the summer of 2001 (Petersen and Hayes 2004). The virus has been associated with thousands of avian mortalities since its initial introduction to North America, with significant impacts to highly susceptible species. For example, Caffrey et al. (2005) reported an estimated 72% decline in an American crow (*Corvus brachyrhynchos*) population after the first year of WNV exposure; similarly, four Greater Sage-Grouse (*Centrocercus urophasianus*) populations experienced a 25% reduction in late-summer survival upon the arrival of WNV (Naugle et al. 2004). Such high mortality rates are probably due to the virulence of the WNV strain introduced into North America, coupled with a lack of

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immunologic resistance that might have been provided by previous exposure to other closely related flaviviruses (Brault et al. 2004, Peterson et al. 2004).

Mechanisms that cause variation in WNV prevalence are not well understood, but recent work indicates that changes in host community composition can influence patterns of viral transmission. Specifically, high host species diversity can lower the transmission of some vector-borne diseases if less competent reservoir hosts dilute pathogen transmission between vectors and highly competent hosts (a mechanism termed the "dilution effect"; Ostfeld and Keesing 2000, Allan et al. 2003, Ezenwa et al. 2006). Passeriformes, an order that includes most songbirds, represent highly competent hosts for WNV (Komar et al. 2003). However, the ability to contract, amplify, and transmit the virus varies greatly among bird species (Komar et al. 2003, Marra et al. 2004, Gibbs et al. 2006*a*). If habitat changes associated with urbanization function to lower host species diversity and also increase the relative abundance of key hosts, then WNV transmission could be higher than expected at urban sites.

Vector feeding preferences will also affect the dynamics of this multi-host arbovirus. This was demonstrated by Kilpatrick et al. (2006), who showed that American Robins (*Turdus migratorius*), relatively uncommon in their sample population, accounted for a large fraction of mosquito blood meals in the Washington, D.C., USA area. Shifts in vector population dynamics associated with increasing breeding sites or warmer microclimates in urban areas could increase exposure to West Nile virus among birds and humans inhabiting urban environments (Epstein 2001, Campbell et al. 2002).

As a third mechanism, resource provisioning in urban environments (e.g., bird feeders and fruiting plants in residential areas) could improve avian host condition or immune defenses, facilitating host survival following infection (Bradley and Altizer 2007). Thus, the observed frequency of exposed and recovered birds could increase with greater urbanization, not because of differential viral transmission, but owing to differential host recovery or tolerance of infection.

In this study, songbird populations were sampled along an urban–rural gradient to evaluate how West Nile virus antibody prevalence in natural avian communities covaried with urban land use in Atlanta, Georgia, USA, a rapidly growing metropolitan area. Urban sprawl in this area is associated with the net loss of 133 acres (~54 ha) of forest each day (American Forests 2001), and a recent state-wide survey of wild songbirds in Georgia demonstrated a weak positive association between WNV antibody prevalence in songbirds and urban/suburban land use on a broad spatial scale (Gibbs et al. 2006*b*). We also investigated the role of host age, nest type, diet, and taxonomic family in explaining variation in WNV antibody prevalence. Finally, a subset of analyses focused on patterns of antibody prevalence and body condition across the urban–rural gradient in Northern Cardinals (*C. cardinalis*), an abundant species in the southeastern USA. Northern Cardinals have been shown to be competent hosts of WNV in studies of captive birds, and display significant tolerance to the infection, as evidenced by the high seroprevalence rates observed in wild populations (Komar et al. 2005, Gibbs et al. 2006*a*).

Methods

Site selection and characterization

Between April and August of 2004–2006, wild songbirds were captured and sampled at 14 sites in and around metropolitan Atlanta, Georgia (Fig. 1). Sites were chosen to reflect variation in land use (residential, commercial, or recreational), human presence (e.g., residential areas or nature preserves), and distance from the city's center (Table 1). Selection was also based on landowner permission, accessibility, and the presence of woody vegetation to facilitate the capture of birds using mist nets. Birds were captured within a 50×50 m area in the center of each site, and adjacent sites were separated by a minimum of 1 km. The degree of urbanization at each site was evaluated using a 44-class land use map of Georgia with 30×30 m resolution, developed by NARSAL (the Natural Resources Spatial Analysis Laboratory, University of Georgia, 1998). The coverage area of each land use class was calculated at a radial distance of 500 m from the center of the sampling area (see Appendix B: Fig. B1b), using spatial analyst in ArcMap 8.3 (ESRI, Redlands, California, USA) and V_LATE (Vector-based Landscape Analysis Tools Extension, *available online*).[5] From these data, four variables were extracted: impervious (i.e., road or building) surface coverage (m^2), total forested area (m^2), number of forest patches, and total core forest area given a 10-m buffer edge (m^2). The total forest area was divided by the number of forest patches to obtain an average measure of forest continuity, hereafter termed "average forest patch size."

To compare urbanization measures derived from the NARSAL land use map with those from finer resolution aerial images, we obtained digitized orthophoto quarter-quadrangles (DOQQs) compiled in 1999 and provided by the Georgia GIS Data Clearinghouse (*available online*).[6] At the same 500 m radius, land use objects were digitized using a GIS database in ArcInfo 8.3 (ESRI, Redlands, California, USA). For this classification, building, road, forest canopy, yard, water, and pasture were delineated (see Appendix B: Fig. B1c). Each map was ground-truthed by recording the geographic coordinates of land use boundaries using a hand-held GPS unit (Magellan Pro-Tracker, Santa Clara, California, USA) to account for any digitizing errors or recent development that would not be observed

[5] ⟨http://www.geo.sbg.ac.at/larg/vlate.htm⟩
[6] ⟨http://www.gis.state.ga.us⟩

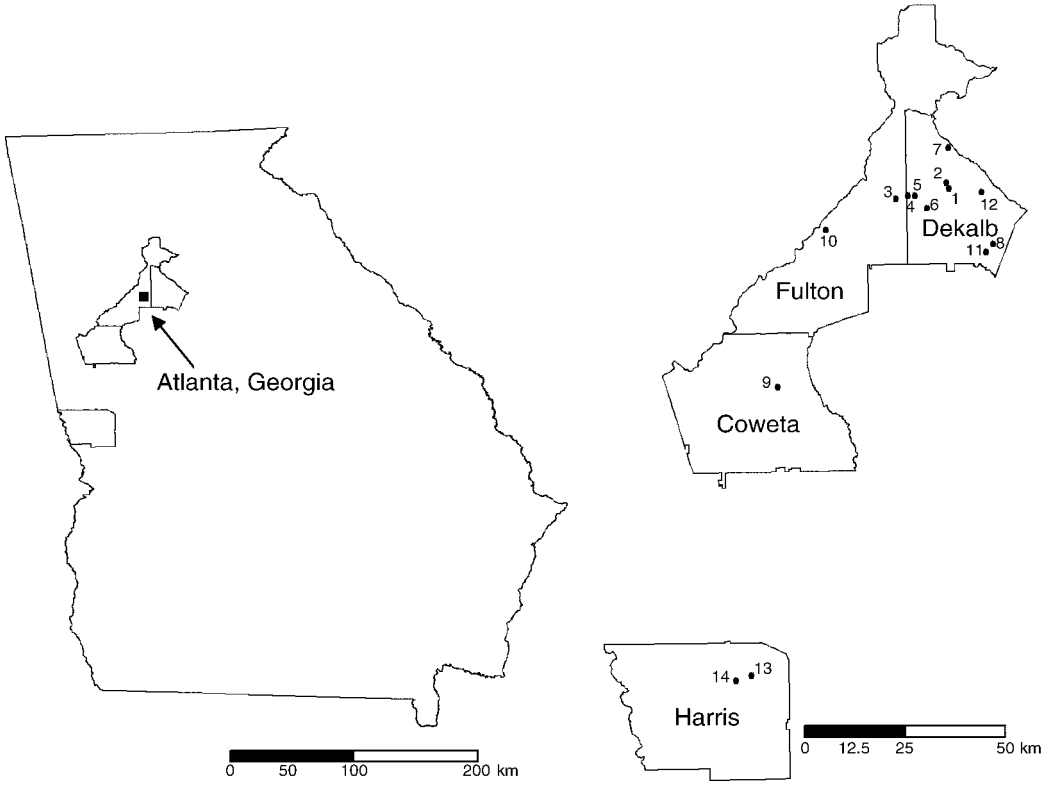

FIG. 1. Map showing the geographic location of the 14 study sites (in the enlarged counties on the right) and the location of the sample area within Georgia, USA (left). The two most distant sites are separated by 132 km, and the minimum distance between sites is 1 km.

on the DOQQ. As previously described, V_LATE was used to obtain three variables: impervious surface coverage (m^2), total core forest area (m^2), and average forest patch size. Digitizing and ground-truthing of DOQQs provides detailed information but is very time consuming. Therefore, we compiled DOQQ data from seven of the 14 sampling sites (4, 6, 7, 8, 10, 11, and 13) chosen to represent a range of high to low urbanization, and used these data to examine correspondence with measures derived from the NARSAL land use map.

Finally, human population density was estimated for each site using 2000 census data available at the finest scale level of the census block group. Here, it was assumed that the human population was evenly distributed throughout the block group and we used ArcMap to calculate the proportion of each block group

TABLE 1. Quantitative measures of urbanization (urban score, described in *Methods*) and habitat description for the sampling locations around Atlanta, Georgia, USA.

Site ID	Urban score	Category	Site description
1†	1.419	U	dense urban residential area
2	1.391	U	forest fragment located inside dense urban area
3†	1.203	U	border of central city park and downtown residential neighborhood
4	0.983	U	Emory University campus annex with low use
5	0.540	U	Emory University main campus
6	0.481	U	forest fragment in a residential neighborhood with little active management
7	0.205	U	urban neighborhood and park mix
8†	−0.400	NU	relict farmland preserved by county park department; low-density residential
9	−0.708	NU	suburban residential neighborhood
10†	−0.711	NU	forest fragment preserved by local climbers as a bouldering site
11	−0.963	NU	relict farmland preserved by county park department
12	−0.998	NU	habitat set aside for songbirds within managed park
13	−1.219	NU	rural, low-density; farm and residence mix
14†	−1.223	NU	rural, low-density; farm and residence mix

Note: Key to abbreviations: U, urban; NU, nonurban.
† Sites omitted in the analyses restricted to sites with >30 samples.

comprising a site. This proportion was then multiplied by the population in the census block. Because most sites contained portions of 2–3 census block groups, population estimates were summed to provide a single estimate of human population density for each sample site.

Field sampling and data collection

Wild birds were sampled 3–6 times per year at each site (see Fig. 1) during 2004, 2005, and 2006. We captured animals during the breeding season (April–August) and sampled each location for 2–3 consecutive days every 6–8 weeks (Fig. 1). Using 30-mm mesh mist nets 6 m and 9 m long, (Avinet; Dryden, New York, USA) open from dawn until late morning, we trapped a total of 802 birds; blood samples from 534 individuals were collected across all sites and years (details are provided in Appendix A).

Species identity, age, and sex were determined following Pyle (1997); age was assigned as juvenile (hatch-year) or adult (after-hatch-year) based on plumage, gape, and skull ossification; we also examined adults for the presence of a brood patch or cloacal protuberance (indicative of breeding status). For each individual, body mass to the nearest 0.1 g and length of the right tarsus to the nearest 0.1 mm were measured. Two categorical measures of condition were also noted. The pectoral muscle development around the carina (or breastbone) was scored following Gosler (1991) as: 1, severely sunken pectoral muscle; 2, sunken pectoral muscle; 3, pectoral muscle even with the carina; or 4, pectoral muscle development beyond the carina. The amount of visible subcutaneous fat in the furculum was similarly scored (following Hartup et al. 2001) as: 0, no fat visible; 1, furculum one-third full; 2, furculum one-third to two-thirds full; 3, furculum full; or 4, fat bulging from the furculum. Data on species nest location (ground, brush, cavity, or tree canopy), and primary diet (seed or insect) were later recorded based on species accounts from the Birds of North America periodical series (American Ornithologists' Union, 1992–2002). Northern Cardinals were banded using U.S. Fish and Wildlife Service metal bands; all other species were color-marked to track recaptures.

From birds weighing >10 g, 50–100 μL of blood was collected by ulnar (wing) venipuncture. Blood samples were maintained at ambient temperature for at least 10 minutes and were then kept cool until returning to the lab. The samples were then centrifuged at 10 000 *g* (98 066 m/s^2) for 10 minutes and both serum and erythrocytes were stored at −70°C until the WNV antibody assay was performed.

Assays to detect antibodies to WNV were conducted using an epitope-blocking ELISA (enzyme-linked immunosorbent assay) developed by Blitvich et al. (2003). The assay employs the flavivirus-specific MAb6B6C-1 and the WNV-specific monoclonal antibody (MAb)3.112G to distinguish WNV from other flaviviruses including St. Louis encephalitis. An inhibition value of ≥30% was considered to indicate the presence of viral antibodies. All tests were repeated and samples too small to perform replications were excluded from the analyses. Previous work demonstrated that the assay was valid across a wide range of avian taxa and results were in good agreement with those from plaque-reduction neutralization tests, PRNT (Blitvich et al. 2003). In one study involving Rock Pigeons (*Columba livia*), ELISA results detected circulating antibodies at least 45 weeks postinfection (Gibbs et al. 2005).

Analysis of land use variables

All land use data were transformed using the *z* score ($[x - x_{\mu}]/x_{SD}$; Gotelli and Ellison 2004) to place measures on the same proportional scale prior to analysis. A principal component analysis (PCA) was conducted using JMP 4.0.4 (SAS Institute, Cary, North Carolina, USA) to derive a composite measure of urbanization for each site (Table 1). The results from all unique four-, three-, and two-way combinations of land use variables (impervious surface coverage, total core forest area, and average forest patch size) and human population density were evaluated. Because average forest patch size is the ratio of total forested area and number of forest patches, we did not include these latter two variables separately in the analysis. The PCA with the highest variable loadings, highest percentage of variance described, and best fit to the seroprevalence data was retained. Hereafter, this variable is referred to as the "urban score." Principal component analyses were performed separately for the Georgia land use map and the DOQQ-derived data, and we used Spearman's correlation to compare urban score variables obtained from these two approaches.

Analysis of antibody prevalence, host condition, and urbanization

To investigate the association between antibody prevalence and urban score, we used generalized linear models (glm) with binomial errors in R 2.2.0 (*available online*).[7] The full model included host age, year and month of sampling, urban score, and all relevant two- and three-way interactions as explanatory variables. The minimum adequate model was obtained by removing nonsignificant terms, starting with the highest order interactions, and model comparison was performed based upon *P* values and Akaike's information criterion (following Crawley 2002). Two separate sets of analyses were conducted, first using data from all 14 locations, and second using data from nine sites where >30 individual birds had been tested (sites removed: 1, 3, 8, 10, and 14). Taxonomic (species and family) and ecological (nest type and primary diet) associations with antibody prevalence were tested separately using an analysis of deviance with binomial errors,

[7] ⟨http://www.r-project.org⟩

treating site as a categorical variable (urban or nonurban; Table 1). Only species with 10 or more individuals (American Robin, Carolina Wren (*Thryothorus ludovicianus*), Eastern Towhee (*Pipilo erythrophthalmus*), Tufted Titmouse (*Baeolophus bicolor*), Gray Catbird (*Dumetella carolinensis*), House Finch (*Carpodacus mexicanus*), and Northern Cardinal), and families with 15 or more individuals sampled (Cardinalidae, Emberizidae, Mimidae, Paridae, Troglodytidae, Turdidae) were included in the taxonomic comparisons. All models were checked for overdispersion, and models where quasibinomial distributions were required are noted in the results (Crawley 2005). West Nile Virus antibody proportions reported in the text were compared using a binomial proportions test with 95% CI.

Because Northern Cardinals were well represented in the data set and accounted for over one-half of the seropositive samples from all birds (described in *Results*), we conducted a final set of analyses focused on antibody prevalence, body condition, and urban land use for this species. To develop a composite measure of individual body condition, a PCA was performed using the ratio of body mass to tarsus length, pectoral muscle development score, and subcutaneous fat score. The first principal component was retained as a measure of individual condition. An analysis of covariance in JMP 4.0.4 was used to examine the association between individual condition in Northern Cardinals, host age, exposure to WNV (presence of antibodies as a fixed factor), urban land use (as a continuous covariate), and all two- and three-way interactions. Model simplification was performed as described previously.

Results

Land use characterization

Urban score values were derived from a PCA of two variables, human population density and average forest patch size, explaining 85.2% of the variation in land use measures between sites. Individual variable loadings were human population density (0.707) and average forest patch size (–0.707). Therefore, a high urban score represents a site characterized by high human population density and low average forest patch size (Table 1). Urban scores obtained from analysis of DOQQ-derived data were highly correlated with those from the Georgia land use map (Spearman's $r = 0.93$, $P = 0.007$), supporting use of the NARSAL Georgia land use data for further analyses.

WNV antibody prevalence

From the 802 birds captured throughout the course of the study, 23 individuals (2.9%) were recaptured and only data from the first sample obtained are included in this study. Of the 534 samples collected for testing, assay results were obtained from 499 samples. The remaining 35 samples were excluded either because they were too small to perform replicate tests or results were inconclusive. A total of 73 samples (14.6%) were positive for antibodies to WNV (see Appendix A). Among the 14 study sites, WNV antibody prevalence ranged between 6.3% ($N = 43$ birds) and 30.8% ($N = 52$ birds). There was no significant difference in WNV antibody prevalence by month or year of sampling. However, WNV antibody prevalence was higher among adult birds (18.3%) relative to juveniles (10.8%; $\chi^2 = 7.0429$, $P = 0.008$). Of adult birds, Northern Cardinals represented 37.0% of the total sample population ($N_{ad} = 257$ birds) and were the only species sampled across all 14 sites. Antibodies to WNV were detected in 18.8% of all sampled Northern Cardinals ($N = 170$ birds), and 27.4% of all adult Northern Cardinals ($N = 95$ birds). Adult Northern Cardinals accounted for 55.3% of all seropositive samples obtained from adult birds ($N = 47$).

WNV antibody prevalence and urban land use

Model simplification showed that urban score and host age were strong predictors of seroprevalence, but the two-way interaction between these factors was not significant (for urban score, $\chi^2 = 43.994$, df = 1, $P = 0.006$; for age, $\chi^2 = 36.512$, df = 1, $P = 0.006$). Because WNV antibody prevalence differed significantly between age groups, the strength of the association between urban score and WNV antibody prevalence was examined separately for each age class. West Nile virus antibody prevalence in adult birds increased with higher urbanization ($\chi^2 = 14.306$, df = 1, $P = 0.003$, $R^2 = 0.77$), but no relationship was detected between urban score and antibody prevalence in juveniles ($\chi^2 = 21.4596$, df = 1, $P = 0.251$, $R^2 = 0.89$). When the statistical model was repeated using only data from sites with >30 samples, P values and fit of the regression lines were similar for the adult population, but improved considerably for juveniles (for adults, $\chi^2 = 7.2738$, df = 1, $P = 0.005$, $R^2 = 0.72$; for juveniles, $\chi^2 = 8.2910$, df = 1, $P = 0.064$, $R^2 = 0.76$; Fig. 2).

Sites were categorized as urban (positive urban score) and nonurban (negative urban score) to compare observed patterns in WNV antibody prevalence by host species, taxonomic family, nest location, and diet. Overall, antibody prevalence in urban sites (18.5%) was higher than at nonurban sites (9.6%; $\chi^2 = 6.832$, df = 1, $P = 0.009$). Each species with ≥ 10 sampled individuals and each family with ≥ 15 sampled individuals (see *Methods*) were compared to all others combined to test for taxonomic associations with WNV antibody prevalence. Northern Cardinals showed significantly higher seroprevalence than all other species combined ($\chi^2 = 4.763$, df = 1, $P = 0.03$) and, similarly, Cardinalidae differed from all other families combined ($\chi^2 = 5.049$, df = 1, $P = 0.0046$). No other comparisons of WNV antibody prevalence among different species or families were statistically significant. The slope and strength of the relationship between urban score and antibody prevalence for adult Cardinals was similar to the relationship observed among all other bird species (Fig. 3; $\chi^2 = 5.767$, df = 1, $P = 0.016$ for Northern

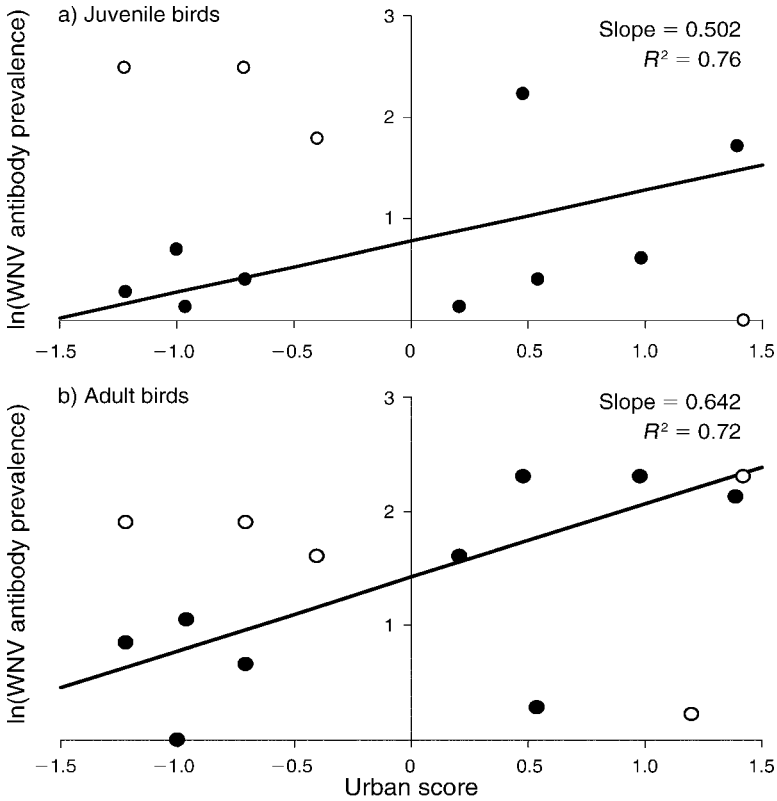

FIG. 2. Relationship between urban score and antibody prevalence of West Nile virus infections in songbird populations by age. To linearize the proportional data, antibody prevalence is presented as the logit transformation of seropositives (ln[seropositive/seronegative]), and values were standardized to zero. Solid circles indicate sites where >30 individuals were sampled, and open circles indicate sites that were removed from a subset of the analyses due to low sample sizes. Regression lines are derived from the estimated slope and intercept values in the generalized linear model using the restricted sample set. Urban score values (see Table 1) were derived from a PCA of two variables: human population density and average forest patch size.

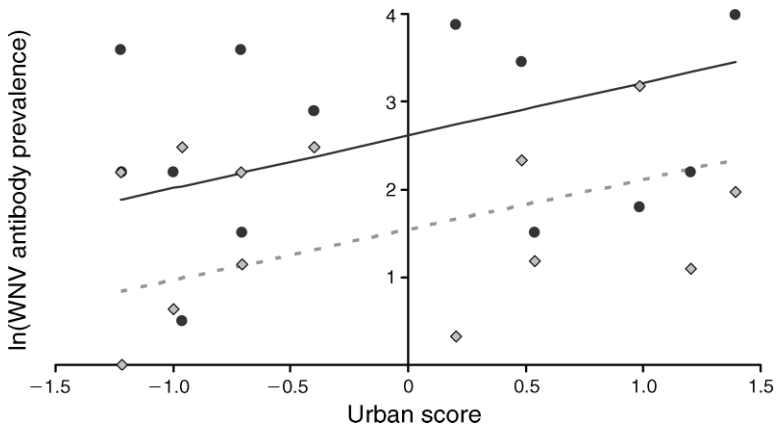

FIG. 3. Comparison of West Nile virus (WNV) prevalence in adult Northern Cardinals (*Cardinalis cardinalis*) and adults of all other songbirds represented by ≥10 samples (see *Methods: Analysis of antibody prevalence, host condition, and urbanization*). Prevalence data were transformed and standardized as described in Fig. 2. Site 1 was removed from the analysis because no adult Northern Cardinals were tested there. Northern Cardinals (solid circles and solid regression line) had significantly higher seroprevalence than other bird species (gray diamonds and dashed regression line), and this difference was observed at all but two sites. The overall relationship between WNV antibody prevalence and urban score was similar for Northern Cardinals (slope = 0.603, $R^2 = 0.28$) and all other bird species (slope = 0.574, $R^2 = 0.35$).

Cardinals; $\chi^2 = 5.147$, df = 1, $P = 0.023$ for all other species; models were run using the quasibinomial distribution to account for overdispersion). Finally, neither nest location nor primary diet explained variation in WNV antibody prevalence across all sites or within site categories.

The association among WNV antibody prevalence, urban land use, and individual body condition was examined in Northern Cardinals using data from the nine sites where >30 individual birds were sampled. The first principal component from a factor analysis of the ratio of body mass to tarsus length and the subcutaneous fat score explained 62.6% of the total variance. Birds with high condition scores had more subcutaneous fat and weighed more relative to their body size than birds with negative scores; component coefficients were subcutaneous fat (–0.707) and mass : tarsus length (0.707). Because visual inspection of the data suggested a nonlinear relationship between urban score and the condition of Northern Cardinals sampled at each site (Fig. 4; condition was greatest at sites of intermediate urbanization), we included both urban score and (urban score)^2 in the full model, together with antibody status, age, and all relevant interactions. Model simplification provided no support for antibody status, age, or two-way interaction effects on condition, but showed a significant relationship between host body condition and the squared term for urban score ($F_{2,140} = 3.27$, $P = 0.014$).

Discussion

The prevalence of antibodies against West Nile virus in wild songbird populations increased with greater measures of urbanization across locations sampled around Atlanta, Georgia, USA. Among adult birds, seroprevalence was nearly 2.5 times higher at urban sites than at nonurban sites. This association was not significantly affected by month or year of sampling, although adult birds were more likely to have WNV antibodies than juveniles. The effect of host age probably resulted from the limited sampling period each year, because many juvenile birds were sampled before the end of the peak transmission period. Moreover, because we tested for antibodies to West Nile virus rather than current infection, and because antibodies to WNV can be long-lasting (Gibbs et al. 2005), greater antibody prevalence among adult birds would also be expected due to longer exposure times.

Northern Cardinals (*Cardinalis cardinalis*), the most commonly sampled host species, had higher WNV antibody prevalence than all other species combined. This is consistent with previous studies conducted in the southeastern United States (Godsey et al. 2005, Komar et al. 2005, Gibbs et al. 2006*a*). The Northern Cardinal's ubiquitous occurrence along the urban–rural gradient and high abundance points to their utility as a surveillance species, as suggested in Gibbs et al. (2006*a*). The role of Northern Cardinals in WNV epidemiology, however, is not well understood. High WNV antibody prevalence rates observed across several studies indicate that cardinals may tolerate infections with WNV more successfully than other avian species (e.g., Caffrey et al. 2005). Komar et al. (2003, 2005) concluded that both Northern Cardinals and House Sparrows (*Passer domesticus*) were important amplifying hosts in southern Louisiana, based on species abundances, exposure rates, and a competence index derived from experimental infections (the product of susceptibility, infectiousness, and the duration of infectiousness). Moreover, Apperson et al. (2002) observed that American Robins, Northern Cardinals, and Northern Mockingbirds (*Mimus polyglottus*) accounted for a high percentage of mosquito blood meals around Queens, New York (16%, 13%, and 13%, respectively). In contrast, Kilpatrick et al. (2006) found that Northern Cardinals were poorly represented in mosquito blood meals around the Washington, D.C. area. Collectively, these studies suggest that the role of Northern Cardinals in WNV transmission could vary over space and time, and point to the need for more data on the contribution of different bird species to WNV transmission.

The positive association between WNV antibody prevalence and urban land use observed in this study could arise from several mechanisms. First, several previous studies of North American metropolitan areas demonstrate declines in avian species diversity with urban land use (Beissinger and Osborne 1982, Green and Baker 2003, Chace and Walsh 2006), combined with a dominance of nonnative and anthropophilic species (Hennings and Edge 2003, Crooks et al. 2004). Because viral amplification and transmission are known to vary among avian species (Komar et al. 2003), host communities characterized by high species richness could dilute the influence of highly competent hosts. This process, termed the "dilution effect," has been proposed as a major cause of variation in Lyme disease occurrence in response to suburban land use in the northeastern United States (Schmidt and Ostfeld 2001, LoGuidice et al. 2003, Keesing et al. 2006); its significance for other pathogens in urbanized areas, however, remains unknown (Bradley and Altizer 2007). Ezenwa et al. (2006) found evidence for a dilution effect in West Nile virus transmission by linking nonpasserine avian species richness to reduced infection levels in mosquito vectors and fewer human cases in Louisiana, USA. If host species diversity affected patterns observed in the present study, we would expect to find lower species diversity and greater dominance of Northern Cardinals with increasing urbanization. Thus, examining measures of host diversity in conjunction with WNV antibody prevalence in avian species represents an important goal for future work.

Changes in vector ecology with increasing urbanization could also affect viral exposure among wild songbirds. Breeding in man-made water containment systems (such as borrow pits, wastewater treatment

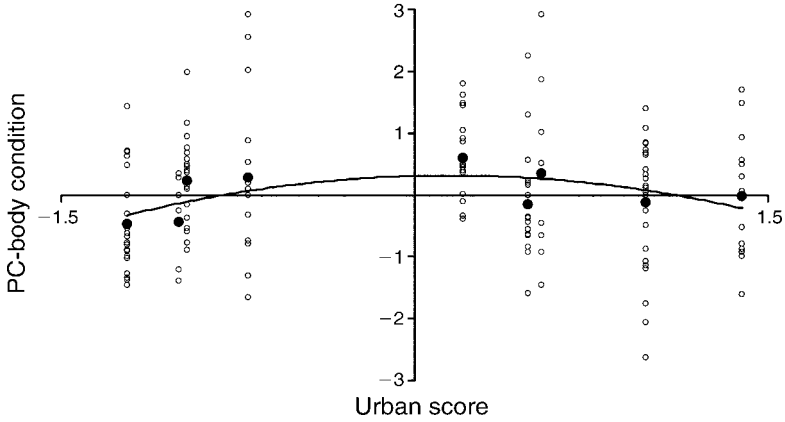

FIG. 4. Relationship between urban score and a composite measure of body condition (derived from PCA based on ratio of body mass to tarsus length, pectoral muscle development score, and subcutaneous fat score) in Northern Cardinals at the study sites where >30 individuals were sampled. Open circles represent individual condition values, and solid circles indicate the average condition of individuals sampled at each site. The regression line shown is derived from average condition values from the full model (including urban score and [urban score]^2) and is fitted to the following relationship: slope for urban score = 0.123; slope for [urban score]^2 = −0.344, $R^2 = 0.41$.

plants, and sewers), *Culex* spp. mosquitoes are well adapted to human-dominated environments, and an increased abundance of mosquitoes could lead to higher avian seroprevalence in urban environments. Indeed, this has been suggested as a likely cause behind urban foci in recent WNV outbreaks in humans in the United States (Epstein 2001, Campbell et al. 2002). Warmer urban microclimates could also favor higher rates of virus replication within the vector and more efficient transmission to susceptible hosts (Reisen et al. 2006).

Finally, habitat changes associated with urban landscapes could affect a host's tolerance to WNV infections. Although urbanization can increase stress levels and reduce immunocompetence in some host species, abundant and consistent food resources available to urban-adapted wildlife may improve host recovery or survival following infection (Bradley and Altizer 2007). Because this study examined only WNV antibody prevalence, it is not possible to exclude the possibility that recovery, rather than exposure, varies with the intensity of urban development. Importantly, we observed significant variation in individual Northern Cardinal condition along the urban gradient, with greatest measures of body condition at sites with intermediate levels of urbanization. Because these sites are primarily suburban or residential, a likely explanation is that supplemental food sources (in the form of bird feeders and fruiting vegetation) are also highest at these sites, leading to increased foraging success and continuous access to food throughout the seasons. If high nutrition or reduced energy expenditure during foraging increases the body condition of birds at these sites, their tolerance to infection and survival following exposure might increase. To the authors' knowledge, there are no published studies that report on the impact of nutritional supplementation to WNV tolerance and recovery in wild birds. With respect to this field study, increased body condition among Northern Cardinals at sites with intermediate urbanization might partially explain the pattern of low WNV antibody prevalence at the least urbanized sampling locations.

We found no evidence of yearly changes in average WNV antibody prevalence, counter to Gibbs et al. (2006*a*), who demonstrated increasing WNV seroprevalence in wild avian hosts from 2000 to 2004 throughout the state of Georgia. This is not surprising, however, because data reported in Gibbs et al. (2006*a*) spanned the period of virus introduction (with human cases starting in Atlanta in 2001). Increasing prevalence of WNV antibodies in the primary hosts was observed during the establishment phase of the pathogen, whereas samples in the current study were collected several years after viral introduction.

Finally, our analysis of land use characteristics indicates that the composite measure of urbanization derived from a previously developed map of Georgia was an accurate and efficient manner of land use characterization. In comparison to the more labor-intensive method of digitizing and ground-truthing orthophotographs, it appears that the coarser scale land use map was an accurate reflection of land use at the 500-m scale. Such data could then be applied over larger geographic areas to create predictive risk assessment maps of WNV antibody prevalence in wild songbird hosts.

The impact of urban landscapes on infectious disease dynamics within wildlife hosts is significant for wildlife management and public health policies (Bradley and Altizer 2007). With respect to multi-host generalist pathogens, the presence of more competent reservoir hosts (and factors that increase their tolerance to infection) can contribute to the extirpation of vulnerable

host species that suffer high mortality rates following infection (Woolhouse et al. 2001, Naugle et al. 2004, de Castro and Bolker 2005). Because the majority of emerging human infectious diseases are zoonotic (Taylor et al. 2001), determining how urban landscapes influence wildlife infectious disease will become increasingly important for predicting human disease risks as well. Our study represents an important step toward understanding the dynamics of WNV at a regional scale in a rapidly growing metropolitan area by demonstrating that WNV antibody prevalence in the avian community was strongly associated with urbanization. Further studies to identify the mechanisms driving this pattern are critical for understanding the dynamics of this and other complex multi-host infectious diseases.

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APPENDIX A

Summary table of bird species represented in the sample population across the three-year study period (*Ecological Archives* A018-036-A1).

APPENDIX B

The (a) digital orthophoto quarter-quad, (b) Georgia land use map, and (c) ground-truthed DOQQ for study site 8 (*Ecological Archives* A018-036-A2).

Effectiveness of Mosquito Traps in Measuring Species Abundance and Composition

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ABSTRACT Mosquito species abundance and composition estimates provided by trapping devices are commonly used to guide control efforts, but knowledge of trap biases is necessary for accurately interpreting results. We tested the hypothesis that commercially available traps (Mosquito Magnet–Pro, the Mosquito Magnet–X) would be significant improvements over the CDC Miniature Light Trap with respect to abundance, species diversity, and measures of recruitment in a wooded area of the Bronx Zoo in New York City, NY. The Mosquito Magnet–Pro collected significantly more mosquitoes (n = 1,117; mean per night, 124 ± 28.3) than the CDC Miniature Light Trap (n = 167; mean per night, 19 ± 5.5). The Simpson's diversity index was greatest for the Mosquito Magnet–Pro. A CDC light trap from a simultaneous surveillance project was located 15 m away and used as a control trap to test for significant differences in mosquito counts on nights with or without the experimental traps. There were no significant differences between nights, indicating the test traps did not recruit beyond 15 m. The traps differed significantly in abundance, but they had similarly limited sampling areas. Measured differences in abundance were independent of differences in diversity. This study highlights how differences between traps might affect species abundance and composition estimates.

KEY WORDS mosquito, traps, surveillance, species diversity, trap area

Mosquito species abundance and composition estimates can be used to target disease prevention efforts by providing information regarding the spatial distribution of vector species. Trapping, as a means to monitor mosquito populations, is an integral component of surveillance efforts, yet standard techniques for interpreting the results are lacking (Downing 1976, Jensen et al. 1994). Trap design (e.g., Counterflow, downdraft), placement (e.g., height above ground, time of day), location (local environment, habitat specificity of mosquito), and use of attractants (e.g., light, CO_2, octenol) influence mosquito abundance estimates (Kline 1999, Mboera et al. 2000, Burkett et al. 2001, Anderson et al. 2004). These factors affect the species, number, and reproductive status (parity) of mosquitoes captured (Reisen et al. 1999, 2000; Mboera et al. 2000). Knowledge of trap biases is essential when deciding what traps to use, where they are to be deployed, and how to interpret the results. Recognition of these biases is especially important when modeling mosquito species distributions and designing mosquito-borne disease surveillance programs.

In this study, we tested the hypothesis that commercially available traps would show significant differences from a standard surveillance trap with respect to differences in abundance, species diversity, and measures of recruitment. Species abundance is the most commonly reported trap attribute. However, understanding the mosquito species community composition is more complex than basic species abundance measures such as richness. Therefore, this study also evaluates species diversity among the three trap types. Finally, the study tests whether results can be explained by active recruitment of mosquitoes to the collection site or if traps capture mosquitoes that are fortuitously present at the trap site. The findings presented here are salient when collecting and interpreting mosquito surveillance data. These additional facets of surveillance trap data are germane to modeling mosquito species distribution and abundance.

Materials and Methods

This study of trap effectiveness was nested in a larger surveillance study occurring at the Bronx Zoo in

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New York City, NY, in collaboration with the Wildlife Conservation Society. In the larger study, 12 CDC Miniature Light Traps (MLT) were evenly distributed over the park to target mosquito control efforts during a West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) epizootic and to evaluate results. The Bronx Zoo is a 107-ha (265-acre) island of deciduous forest situated in New York City along the Bronx River.

This project used the larger study's trapping infrastructure to compare two commercially available traps to the MLT. The American Biophysics Corporation (North Kingston, RI) markets the Mosquito Magnet as effective control devices for professional and home use. Here, we evaluate how the Mosquito Magnet–Pro (MM-Pro) and its prototype Mosquito Magnet–X (MM-X) compare with the standard MLT with respect to abundance, species diversity, and area of recruitment.

The traps were run at three sites located 15 m equidistant on 9 d between 3 August and 24 August 2000. Traps were systematically rotated between the three sites each night to control for the effect of site. All study sites were located in a continuous deciduous forest with similar ground and canopy cover. One of the surveillance MLTs ≈15 m from the experimental locations was used as a control for the recruitment range comparison.

Traps. The MLT used is manufactured by Clarke Mosquito Control (Roselle, IL) based on the American Biophysics Basic Light Trap. This trap remains relatively unchanged since its 1962 inception (Sudia and Chamberlain 1962). It is powered by a 6-V, 10-ampere-h rechargeable gel-cell battery operating a 0.15-W light and a four-blade fan. The traps were suspended 1.5–2 m above the ground and baited with carbon dioxide provided by a 0.95 liter (1-quart) insulated flask filled with dry ice (Rudolfs 1922).

The MM-X trap differs from the MLT in that it does not use a light as an attractant and uses a different trapping mechanism, namely, Counterflow geometry. Counterflow is generated through the use of two fans simultaneously moving air in opposite directions (for details, see Kline (1999). In brief, a CO_2-enriched plume is released through a center pipe by a motor-driven fan. This center pipe is surrounded by a wider tube through which air is sucked upward into the trap by a second fan into a wide plastic container to hold the mosquitoes attracted to the trap. For this study, the MM-X was baited with dry ice as with MLT and suspended between 1.5 and 2 m above the ground. Two serially connected 6-V, 10-ampere-h rechargeable gel-cell batteries were used to provide the required 12-V power source.

The Mosquito Magnet–Pro (MM-Pro) has a power head that catalytically converts propane into CO_2, heat, and water vapor. These attractants are exhausted outside the trap through a center tube, similar to the MM-X, providing a plume of mosquito attractants. As with the MM-X, two fans work in opposition: one fan provides the exhaust plume of attractants; the other fan provides a counterflow updraft used to capture mosquitoes. It is a freestanding unit with an intake 52 cm above ground level and holds a 9-kg propane tank. In the Northeast, the manufacturer recommends using octenol as an additional attractant, but it was not used for this study to facilitate direct comparisons among the traps.

Traps were set between 8:00 a.m. and 9:00 a.m. with dry ice, propane, fresh batteries, and collection units as necessary. Collections of mosquitoes were recovered 24 h later, frozen on dry ice, and transported to the laboratory in New Haven, CT. Mosquitoes were identified to species using an Industrial Inventions Inc. (Monmouth Junction, NJ), model 1012 electronic chill table under a 10–40× zoom dissection microscope, and then they were frozen at −70°C for later virus isolation as part of a WNV surveillance and control program. Damaged mosquitoes that could not be identified to species were identified to genus when possible or placed into an unidentified class and excluded from this analysis. These exclusions did not significantly differ across the three trap types (Pearson $\chi^2 = 18.0$, $P = 0.324$).

Data Analysis. Count data were tested for normality and transformed using log(count + 1). Differences in abundance were tested by comparing the total number of mosquitoes per trap using Bonferroni analysis of variance (ANOVA) and SAS version 8 software (SAS Institute 2002). The analysis compared trap and site specific differences.

Simpson's diversity index (1-D) was used as a measure of species diversity for each of the three traps (Simpson 1949). Simpson's diversity index was calculated as follows:

$$D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N - 1)}$$

where n is the total number of a particular species (i), and N is the total number of individuals (in this case per trap type).

This index measures the probability that two specimens from a sample will be different species. Simpson's diversity index increases in value as species diversity increases. It is sensitive to the numerical abundance of dominant species (Magurran 1988).

Confidence intervals were calculated to determine whether differences in diversity between the traps was statistically significant (Grundmann et al. 2001):

$$CI = D - 2\sqrt{\sigma^2},\ D + 2\sqrt{\sigma^2}$$

Variance (σ^2) is defined as

$$\sigma^2 = \frac{4}{N}\left[\sum_{i=1}^{s} \pi_i^3 - \left(\sum \pi_i^2\right)\right],$$

where N is the total number of individuals (in this case per trap type), and π_i is the relative frequency of n_i (species)/N (total individuals):

$$\pi_i = \frac{n_i}{N}.$$

Table 1. Mosquito species collected during trap nights

Species	MM-Pro	MM-X	MLT	Total
Aedes vexans	225	189	91	505
Ochlerotatus trivittatus	216	126	17	359
Ochlerotatus triseriatus	164	41	4	209
Culex pipiens	86	67	14	167
Psorophora ferox (von Humbolt)	128	39	0	167
Culex pipiens/restuans	117	13	17	147
Anopheles punctipennis (Say)	59	41	7	107
Aedes/Ochlerotatus (unknown)	74	11	3	88
Culex (unknown)	33	1	6	40
Culex salinarius Coquillett	0	18	2	20
Culex restuans Theobald	6	1	4	11
Aedes intrudens Dyar	3	1	1	5
Aedes canadensis (Theobald)	2	1	0	3
Coquilittidia perturbans (Walker)	2	1	0	3
Culex territans Walker	1	1	0	2
Aedes stimulans (Walker)	1	0	0	1
Culiseta morsitans (Theobald)	0	1	0	1
Orthopodomyia signifera (Coquillett)	0	0	1	1
Psorophora ciliata (F.)	0	1	0	1
Total	1,117	553	167	1,837

Recruitment range was evaluated to assess whether the experimental traps were attracting mosquitoes from >15 m by comparing counts for the control trap (15 m from the test sites) on the nine test nights to the seven intervening nontest nights. This comparison was conducted using the total mosquito count and by the combined *Aedes/Ochlerotatus* species total count and *Culex* species total count using Kruskal–Wallis test (χ^2 approximation) and compared with Wilcoxon rank sum test.

Results

Over the 27 trap nights, 1,837 female mosquitoes were collected, representing 16 species (Table 1). *Aedes vexans* (Meigen) was found in the greatest number for all traps (27% of all mosquitoes trapped; range 20–54%), followed by *Ochlerotatus trivittatus* (Coquillett) (20%, range 10–23%), *Ochlerotatus triseriatus* (Say) (11%, range 2–15%), and *Culex pipiens* L. (9%, range 8–12%). Although the total number of mosquitoes collected was not particularly large, it is the result of an operational mosquito surveillance program during a WNV epizootic; therefore, it is representative of an operational application of these traps.

Abundance. The MM-Pro collected the greatest number of mosquitoes (n = 1,117; mean per night, 124 ± 28.3), followed by the MM-X (n = 553; mean per night, 61 ± 22.7), and the MLT (n = 167; mean per night 19 ± 5.5) (Fig. 1). The ANOVA measure for multiple comparisons showed significant differences in count between the MM-Pro and MLT (ANOVA: $F = 15.76$, $P < 0.01$), but not the MM-X. There were no differences when evaluating count by site (ANOVA: $F = 0.23$, $P = 0.90$) (Fig. 2). This lack of difference indicates the trap, not the location of the trap, yielded significant differences in total mosquito counts.

Species Diversity. The MM-X collected the greatest total number of species (n = 14) followed by the MM-Pro (n = 12) and the MLT (n = 9). However, Simpson's diversity index, which accounts for sampling in the estimation of diversity, was greatest for the MM-Pro (1-D = 0.87, 95% CI: 0.88–0.86) followed by the MM-X (1-D = 0.80, 95% CI: 0.82–0.78) and the MLT (1-D = 0.68, 95% CI: 0.75–0.60). Although the MM-X collected the greatest number of species (species richness), collections from the MM-Pro yielded the greatest measure of species diversity.

Recruitment Range. Analysis of the attraction by experimental traps on total mosquito count by Kruskal–Wallis test (approximation) showed neither significant differences for the total catch comparison on test and nontest nights (Kruskal-Wallis = 0.06, P = 0.81), nor for the species group specific comparisons (*Aedes/Ochlerotatus* species, Kruskal-Wallis = 0.04,

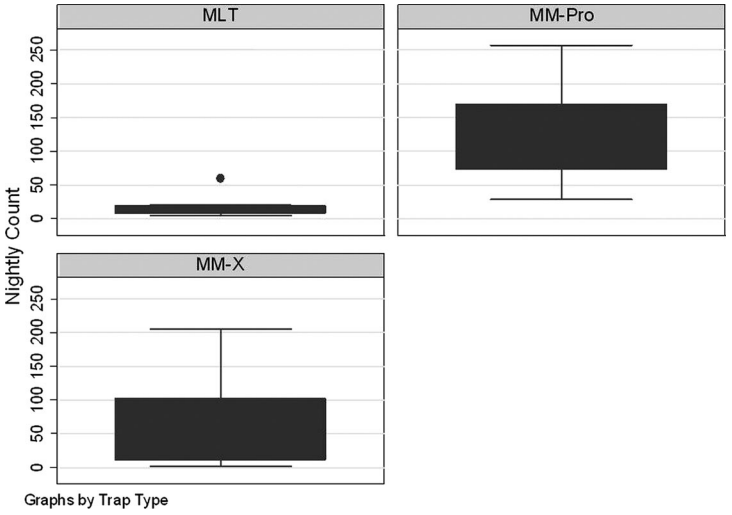

Fig. 1. Box plot of the nightly counts by the three trap types: MLT (n = 167; mean per night, 19 ± 5.5), MM-X (n = 553; mean per night, 61 ± 22.7), and MM-Pro (n = 1,117; mean per night, 124 ± 28.3). There were significant differences in count between the MM-Pro and MLT (ANOVA: $F = 15.76$, $P < 0.01$).

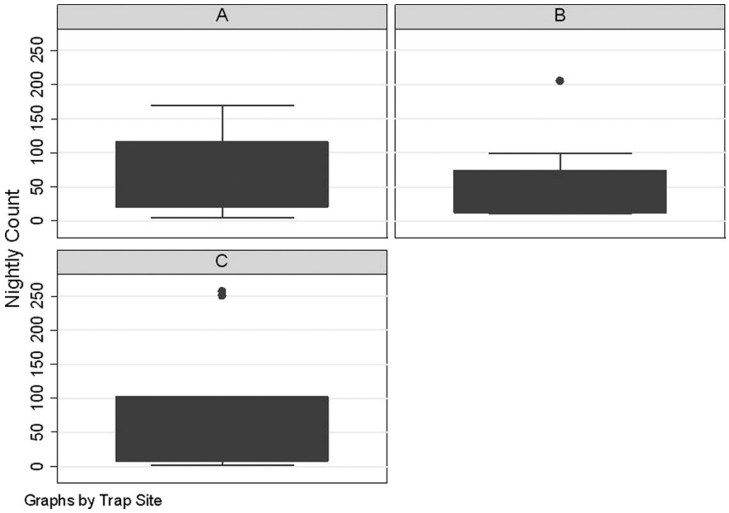

Fig. 2. Box plot of the nightly counts by the three trapping sites shows there is not effect of the site on nightly count (ANOVA: $F = 0.23$, $P = 0.90$).

$P = 0.85$ and *Culex* species, Kruskal-Wallis = 0.24, $P = 0.62$). See Fig. 3 for box plot comparison of nontrap night and trap night counts. Likewise, analysis by Wilcoxon rank sum test indicated no significant difference in total count (Wilcoxon rank sum = −0.20, $P = 0.85$), *Aedes*/*Ochlerotatus* count (Wilcoxon rank sum = −0.15, $P = 0.89$) or *Culex* (Wilcoxon rank sum = 0.44, $P = 0.65$) on test compared with nontest nights. These results indicate that the test traps did not influence results from the surveillance trap located 15 m distant.

Discussion

This study confirms that traps using Counterflow technology collect greater numbers of mosquitoes than standard downdraft traps (Kline 1999, 2002; Burkett et al. 2001). However, traps may be biased toward specific species (Huffaker and Back 1943, Acuff 1976). To understand the composition of the mosquito community this study also examined diversity. The findings support the importance of diversity measures as the trap yielding greatest species richness did not capture the greatest diversity of mosquitoes. These findings are important when comparing across different trap types because greater richness does not necessarily translate into greater diversity.

This study shows that all of the traps are spatially limited with reference to the information they provide about the local mosquito community. To detect what species occur and their relative abundances within an area, we would recommend using replicates of MLTs rather than relying on only one Magnet (MM-X or

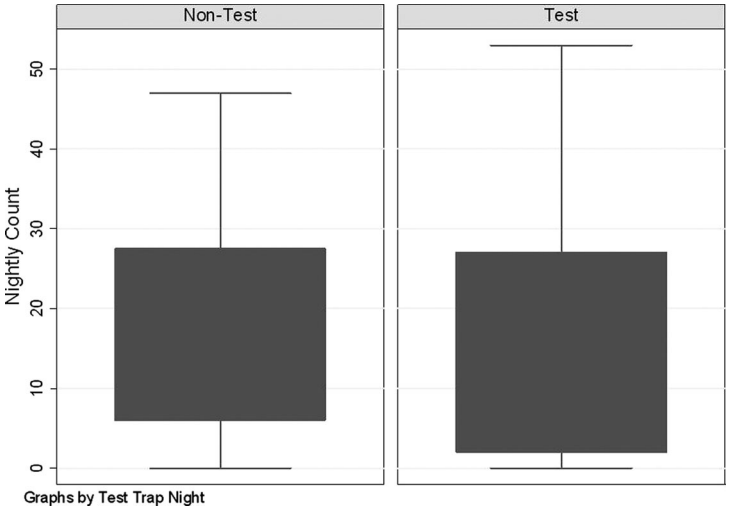

Fig. 3. Box plot of count in the control trap for test versus nontest nights indicates no significant difference for the total catch comparison on test and nontest nights (Kruskal–Wallis = 0.06, $P = 0.81$).

MM-Pro). Although one MLT may yield lower counts, multiple trap locations would offer similar abundance data but with greater spatial resolution. The loss with respect to species diversity might be with rare species and this would have to be considered. However, this duplicative sampling methodology may be more cost effective and would allow for sampling in multiple habitats. Regardless of the trap used, repetition of sites would improve the study design, create greater confidence in the information provided, and traps placed >15 m apart might be independent. An alternative would be to alter the CO_2 output or the wattage of the light attractants, but we view this to be more difficult.

There is increasing use of spatial models to identify high-risk areas for mosquito-borne disease control (Barrera et al. 1999, Brownstein et al. 2003, Diuk-Wasser et al. 2006). With improvements in disease risk models and increasing use of these models to guide intervention efforts, appreciation of the unavoidable errors associated in parameter estimation become more important (reviewed by Liebhold et al. 1993, Rogers and Randolph 2003). Commenting on the information provided by mosquito light traps, Huffaker and Back (1943) stated "the trap is only one component of the picture." Their 64-yr-old comment holds true today, and researchers must ensure that they address the biases inherent in the surveillance data collected. This study quantifies how differences in trap selected can alter the "picture" of mosquito abundance and diversity. Understanding the limitations of mosquito surveillance data is necessary to improve the accuracy of spatial models.

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Colonization of abandoned swimming pools by larval mosquitoes and their predators following Hurricane Katrina

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ABSTRACT: Thousands of flooded swimming pools were abandoned in New Orleans following Hurricane Katrina and provided a natural experiment to examine colonization of a novel aquatic habitat by mosquito larvae and their aquatic predators. We conducted a randomized survey of flooded swimming pools in two neighborhoods in January 2006 and found that 64% contained mosquito larvae, 92% contained predatory invertebrates, and 47% contained fishes. We collected 12,379 immature mosquitoes representing five species, primarily *Culiseta inornata,* and secondarily, the arboviral vector *Culex quinquefasciatus.* Dragonfly nymphs in the families Aeshnidae and Libellulidae were the most common predatory invertebrates collected among a total of 32 non-mosquito invertebrate species. Eleven species of fishes were collected, with *Gambusia affinis* accounting for 76% of the catch. Diversity of fishes in swimming pools was positively correlated with proximity to a levee breach and the fish assemblage found in swimming pools was similar to that found along shorelines of Lake Pontchartrain and drainage canals that flooded the study area. Mosquito larvae were rare or absent from pools containing fishes; however, path analysis indicated that the presence of top predators or abundant competitors may somewhat mitigate the effect of *Gambusia affinis* on mosquito presence. ***Journal of Vector Ecology* 33 (1): 166-172. 2008.**

Keyword Index: Swimming pools, mosquitoes, predators, Hurricane Katrina, path analysis.

INTRODUCTION

Tropical cyclones (hurricanes and typhoons) have profound negative effects on natural and managed landscapes, but they also provide unique ecological opportunities for some species. For example, mosquito larvae and other aerially dispersing insects with aquatic immature stages are very successful at colonizing newly-flooded habitats (Durso and Burguin 1988). Flood-induced changes may increase the populations of hematophagous arthropods but are not generally associated with increases in vector-borne diseases in developed regions (Nasci and Moore 1998). However, flooding often contributes to increased biting by nuisance arthropods and may inhibit recovery and restoration of storm-ravaged landscapes (Shultz et al. 2005). Changes in the type and amount of larval habitat likely contributed to observed changes in size and composition of adult mosquito populations in New Orleans, LA, following the flooding associated with Hurricane Katrina (Caillouët et al. unpublished data).

Aquatic habitats in flooded urban areas include lowlands, depressions, and artificial containers. Another type of novel aquatic habitat created by natural disasters are abandoned swimming pools that are not maintained during the recovery and restoration of urban landscapes. Abandoned swimming pools harbor vectors of human disease (Carlson et al. 2004, Townsend 2005). There is little evidence that pools abandoned following tropical cyclones contribute to human disease risk because residents are typically able to return home and resume maintenance of their swimming pools within a few days. However, what happens to larval mosquito populations when large numbers of residents are unable to return home and flooded swimming pools are necessarily abandoned for months or years?

An opportunity to address this question arose in August 2005 when approximately 80% of New Orleans was flooded as a result of levee failures associated with Hurricane Katrina (Reichardt et al. 2005). Brackish floodwater from Lake Pontchartrain inundated most areas of the city for up to three weeks. Flooding resulted in the creation of novel aquatic habitats that could be colonized by opportunistic mosquito species. Furthermore, prolonged inundation could facilitate dispersal of fishes and other aquatic organisms that resided in Lake Pontchartrain, drainage canals, and recreational lagoons and ponds. We carried out a survey of New Orleans swimming pools about four months after Hurricane Katrina in order to address four related questions: Were mosquitoes using abandoned swimming pools for larval development? Were fishes able to move across the flooded urban landscape and colonize abandoned swimming pools? Did aerially-dispersing insect predators colonize abandoned swimming pools? Finally, were fishes and predatory insects able to regulate larval mosquito populations in abandoned swimming pools?

MATERIALS AND METHODS

Study area

We surveyed swimming pools in the Lakeview and Filmore neighborhoods in North Central New Orleans (Figure 1) (North of I-610, South of Robert E. Lee Blvd., East of the 17th Street Canal, and West of the London Ave. Canal) due to their proximity to breached levees and the relative uniformity of flooding in this part of the city. Using high-resolution aerial imagery captured before Hurricane Katrina (Google Earth 4.0), we identified 270 swimming pools in Lakeview and 166 swimming pools in Filmore. We randomly selected and visited 50 swimming pools from each neighborhood between 18th and 26th January 2006. We could not verify the existence of eight pools (8%) either because of misclassification or because they were above-ground pools that had been removed or destroyed. Seven pools (7%) could not be accessed because of locked gates and another 21 pools were excluded from the survey because they had been drained (12%), cleaned (8%), or covered (1%). We therefore sampled 64 of the 100 pools originally selected, or 32 pools each from the Lakeview and Filmore neighborhoods (Figure 1). The mean surface area of the sampled pools was 50.7 $m^2 \pm 19.1$ SD.

Sampling methods

We used a 300-ml dipper to sample mosquito larvae (O'Malley 1989). Ten dips were collected haphazardly within 2 m of the edge of the pool and the contents of these dips were filtered through a 200 µm sieve and preserved in 70% ethanol. We used long-handled dip nets (mesh size = 6 mm, mouth area = 1400 cm^2) to sample fishes and aquatic invertebrates (Figure 2) large enough to prey upon mosquito larvae (Turner and Trexler 1997). We made 40 1.5-m sweeps in each swimming pool and included all microhabitats (i.e., bottom, mid-water, surface, and near debris). Each sweep sampled about 210,000 cm^3. Organisms were removed from each sweep sample and preserved in 10% buffered formalin. In the laboratory, mosquito larvae (Fox and Wesson 2005) and other invertebrates were identified, enumerated, and categorized by feeding guild (Merritt and Cummings 1996).

Data analysis

Using Global Positioning System (GPS) coordinates, distances were calculated from each of the 32 Lakeview swimming pools to the 17th Street Canal levee breach, which was a likely source of fishes for the Lakeview neighborhood. We used Pearson correlation analysis to test for a relationship between fish species richness and distance to the 17th Street Canal levee breach.

A model food web was created to evaluate the trophic effects of predator combinations on the presence of mosquito larvae (Figure 3A). This model is based on the hypothesis that specialist predators (i.e., fish) are most efficient at reducing prey (i.e. immature mosquitoes). Therefore, the presence of top predators (i.e., belostomatids and nepids) and competitors (i.e., odonates) will indirectly benefit prey by interfering with the direct predatory effects of the specialist predator. This model was applied to the swimming pools in this study, using presence of mosquito larvae as the "prey," Western mosquitofish (*Gambusia affinis*) abundance as the "specialist predators," Odonata abundance as the "competitors," and presence of large predacious bugs (Heteroptera: Belostomatidae and Nepidae) as "top predators" (Figure 3B). Path analysis (AMOS 4.0) was used to derive standardized coefficients to describe the relative indirect and direct effects in this food web. Path analysis uses standardized coefficients from multiple regression analysis to examine the relative strengths of various trophic interactions within food web models (Miura and Takahashi 1988, Manickchand-Heileman et al. 2004). Abundances of Odonata and *G. affinis* were log-transformed to meet assumptions of normality.

RESULTS

Sixty-nine percent (64/92 existing pools) of the swimming pools in our study area remained abandoned four months after Hurricane Katrina. Of these pools, 64% (41 pools) contained mosquito larvae, 92% (59 pools) contained predatory invertebrates, and 47% (30 pools) contained fishes. We collected 12, 379 immature mosquitoes representing five species (Table 1). Eighty-six percent of all mosquitoes collected were identified as *Culiseta inornata*.

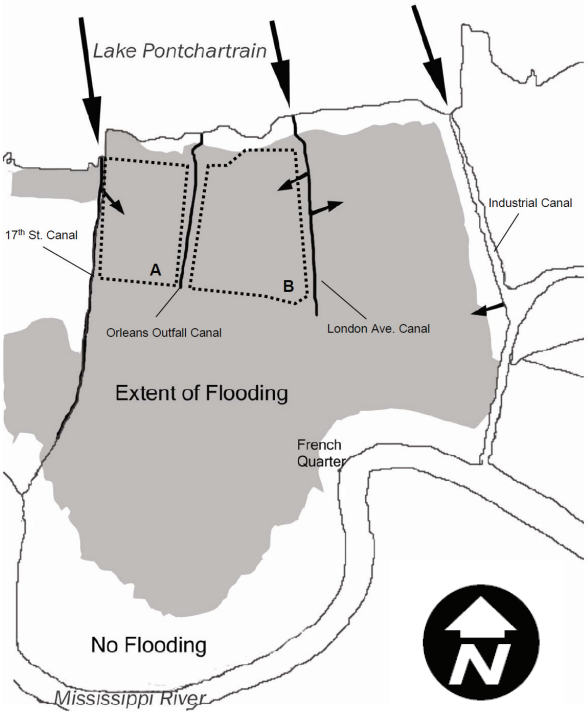

Figure 1. Map of study area and flooding extent, New Orleans, LA. A. Lakeview neighborhood. B. Filmore neighborhood. Large arrows indicate direction of storm surge from Lake Pontchartrain. Small arrows indicate levee breach (source of flooding).

Culex quinquefasciatus (8%), *Cx. salinarius* (4%), *Cx. tarsalis* (3%), and *Anopheles atropos* (0.1%) were also collected.

We collected 1,364 non-mosquito invertebrates representing 16 families (Table 1). Dragonfly nymphs of Families Libellulidae and Aeshnidae were the most common large-bodied invertebrates collected, occurring in 47% and 56% of pools, respectively.

We collected 855 fishes belonging to 11 species (Table 2). The majority (76%) were *G. affinis*. Other numerically dominant fishes included sailfin mollies (*Poecillia latipinnia*, 9%), least killifish (*Heterandria formosa*, 6%), and sheepshead minnows (*Cyprinodon varieagatus*, 5%). Small numbers of inland silversides (*Menidia beryllina)*, rainwater killifish (*Lucania parva)*, diamond killifish (*Adinia xenica)*, bayou killifish (*Fundulus pulverous*), golden topminnows (*Fundulus chrysotus*), naked gobies (*Gobiosoma bosc)*, and introduced Rio Grande cichlids (*Cichlasoma cyanoguttatum*) were also collected. Species richness of fishes in 32 non-maintained Lakeview swimming pools was negatively correlated (r=-0.404, P=0.022) with distance to the 17th Street Canal levee breach.

Path analysis revealed that *G. affinis* had a very large direct negative effect on the presence of mosquito larvae, but that this effect was mitigated somewhat by the presence of top predators and competitors (Figure 3B). We found no direct effects of Belostomatidae/Nepidae presence on Odonata abundance. Additionally, we found no direct effect of Odonata abundance on mosquito presence. A non-significant lack-of-fit test indicates there is agreement between field data and the path model shown in Figure 3B (χ^2= 0.152, 1 df, p=0.696).

DISCUSSION

The floodwaters that covered 80% of New Orleans in September 2005 did not fully recede for over three weeks, and the extent of destruction has kept many homeowners from returning to the city over two years later (Plyer and Bonaguro 2007). In January 2006, immature mosquitoes inhabited 64% of the abandoned swimming pools sampled. Most of these mosquitoes were larvae of the seasonally abundant winter mosquito *Cs. inornata*, which is a laboratory vector of *West Nile virus* (WNV) (Goddard et al. 2002), *Saint Louis encephalitis virus*, and W*estern equine encephalomyelitis virus* (Hammon and Reeves 1943a, 1943b). The New Orleans Mosquito and Termite Control Board estimates that about 5,000 abandoned swimming pools need long-term mosquito abatement (S. Sackett, personal communication). The substantial number of potential vector mosquitoes we collected per pool and the significant number of flooded abandoned swimming pools underscore the public health import of this newly-abundant habitat.

The unexpectedly high density of larval mosquitoes in swimming pools is especially surprising given that this study was conducted in January, a month in which both mosquito activity and arbovirus transmission are typically very low (Michaels et al. 2005). The high productivity of swimming pools in January suggests that, if left untreated, these habitats will be important for both vector and nuisance mosquitoes during warmer months. Though we collected predominantly *Cs. inornata* from the pools, the second most abundant mosquito in our study was *Cx. quinquefasciatus,* the primary vector of WNV in Louisiana (Godsey et al. 2005). It is likely that as 2006 progressed, other mosquitoes, including *Cx. quinquefasciatus,* became more prolific in the

Figure 2. A flooded abandoned swimming pool in the Lakeview neighborhood of New Orleans.

Table 1. Summary data for Hexapods collected from abandoned swimming pools in New Orleans in January 2006. Family groups are presented in bold along with their corresponding total organisms collected (also in bold).

Order	Family	Species		# Organisms collected
Hemiptera	**Belostomatidae**			**38**
		Belostoma lutarium		6
		Belostoma testacium		31
		Lethocercus uhleri		1
	Gerridae	*Gerris canaliculatus*		**4**
	Nepidae			**30**
		Ranatra australis		29
		Ranatra buenoi		1
	Notenectidae			**11**
		Buenoa margaritacea		2
		Buenoa scimitra		2
		Notonecta indica		2
		Notonecta irrorata		5
	Corixidae	*Tricorixa louisianae*		**2**
	Naucoridae	*Pelocoris carolinensis*		**1**
Odonata	**Libellulidae**	*Pantala hymanaea*		**289**
	Aeshnidae	*Anax junius*		**327**
	Coenagrionidae	*Ishnura ramburii*		**174**
Coleoptera	**Hydrophilidae**			**214**
		Tropisternus lateralis nimbatus		81
		Tropisternus collaris		16
		Tropisternus quadristriatus		12
		Tropisternus blatchleyi		99
	Dytiscidae			**238**
		Agabus sp.		3
		Cybister fimbriolatus crotchi		5
		Graphoderus sp.		1
		Rhantus sp.		1
		Thermonectes basillaris		223
		Thermonectes ornaticollis		5
	Haliplidae	*Peltodytes sp.*		**12**
Ephemeroptera	**Baetidae**	*Callibaetis sp.*		**17**
Odontomyia	**Stratiomyidae**			**9**
		Catatasima sp.		6
Diptera		*Odontomyia sp.*		3
	Syrphidae	*Eristalis sp.*		**2**
	Ephyridae	*Brachydeutera sp.*		**1**
	Noctuidae	*Archanura sp.*		**1**
	Culicidae			**12,379**
		Culiseta inornata	4th instar	1,570
		Culex quinquefasciatus	4th instar	138
		Culex salinarius	4th instar	64
		Culex tarsalis	4th instar	50
		Anopheles atropos	4th instar	2
		Unknown	Pupae	655
			3rd instar	3,084
			2nd instar	3,799
			1st instar	3,017

Table 2. Summary of data for fishes collected from abandoned swimming pools in New Orleans in January 2006.

Fishes	# Organisms collected	% of collected (all species)	# of Swimming Pools (n = 64)	% of + Swimming Pools	Mean Catch per + Pool*
Gambusia affinis	652	76.3%	16	25.0%	27.7
Poecillia latipinnia	81	9.5%	11	17.2%	2.7
Heterandria formosa	50	5.8%	9	14.1%	1.7
Cyprinodon varieagatus	42	4.9%	6	9.4%	1.4
Menidia beryllina	13	1.5%	1	1.6%	0.4
Lucania parva	8	0.9%	3	4.7%	0.3
Adinia xenica	4	0.5%	1	1.6%	0.1
Fundulus pulvereus	2	0.2%	2	3.1%	0.1
Cichlasoma cyanoguttatum	1	0.1%	1	1.6%	<0.1
Fundulus chrysotus	1	0.1%	1	1.6%	<0.1
Gobiosoma bosc	1	0.1%	1	1.6%	<0.1
Total	**855**				

* Mean Catch per + Pool refers to the average number of fish of each species collected in pools where at least one fish was collected.

absence of the dominant winter mosquito *Cs. inornata*. Such an increase in WNV vector abundance may increase WNV transmission to humans. Indeed members of our research team recently demonstrated a significant increase in West Nile neuroinvasive disease in 2006 in the hurricane-affected region (Caillouët et al. unpublished data).

Fishes, consisting predominantly of *G. affinis*, were observed in about half of the swimming pools sampled. Mosquitofish (*Gambusia* spp.) were used as the first biological method of mosquito control in the early 20th century and have since been distributed worldwide (Bay 1967). The native range of *G. affinis* is the Mississippi River Basin (Ross 2001), and it is fortuitous that this species continues to perform its natural role in the very area where it honed its mosquito hunting skills. Other fishes found in swimming pools are commonly found in Lake Pontchartrain and the drainage canals that flooded the Lakeview area (Duffy and Baltz 1998). No aquarium species were collected, but a single non-native Rio Grande cichlid (O'Connell et al. 2002) was collected. Species richness of fishes was strongly correlated to the proximity of the flooding source (the 17th Street Canal) further implicating the levee breach as the source of fishes in this study.

Insect predators have received a great deal of interest as potential biological control agents due to their ability to rapidly disperse into new habitats (Lacey and Orr 1994, Bay 1974). Of the predatory invertebrates we collected, only odonate larvae appeared abundant enough to have a direct effect on immature mosquitoes. Nymphal odonates are voracious predators of mosquito larvae in controlled settings and small natural habitats (Fincke et al. 1997, Lacey and Orr 1994, Stav et al. 2000, Yanoviak 2004). However, in our study, path analysis concluded that odonate nymphs did not have a significant direct impact on mosquitoes in abandoned swimming pools. Sunahara et al. (2002) proposed that the tendency for odonate nymphs to cling to the walls and bottom of large deep containers may limit their efficacy in controlling mosquitoes. It is likely that the odonate nymphs observed in our study have few encounters with surface-breathing mosquitoes due to the depth and structurally simple walls of swimming pools.

In many cases, the presence of competing predators or top predators has significant non-linear implications for the survival of prey (Wissinger and McGrady 1993, Finke and Denno 2005). Because of the potential for predator-predator antagonisms, trophic relationships should be evaluated prior to manipulating assemblages in biological control efforts (Brodeur and Boivin 2006). Such antagonisms may result in the local extinction of mosquito predators when "top predator" species are also present (Marten et al. 2004), or in "prey switching" in the presence of alternate prey sources (Chesson 1989), or when competitors are present. Members of the Heteropteran families Belostomatidae and Nepidae are piscivorous as well as insectivorous. While these "top predators" did not affect Odonata abundance in this study, they had a large negative effect on *G. affinis* abundance. This resulted in an equally large positive indirect impact on the presence of mosquitoes. However, this indirect effect was less than half the magnitude of the direct effect of *G. affinis* on mosquito presence, suggesting that at the time of this study *G. affinis* were effective in controlling mosquitoes despite the presence of strong top predators.

Because abundance of native fishes had a large negative effect on mosquito abundance even with top predators and competitors present, manual distribution of *G. affinis* was suggested as an effective intervention at the time of this study. Larval control of mosquitoes is an effective, cost-efficient method used to reduce mosquito populations and the diseases they vector (Keiser et al. 2005). Based in part on our findings, the New Orleans Mosquito and Termite Control Board initiated a large-scale program of introducing locally-collected *G. affinis* into abandoned pools throughout

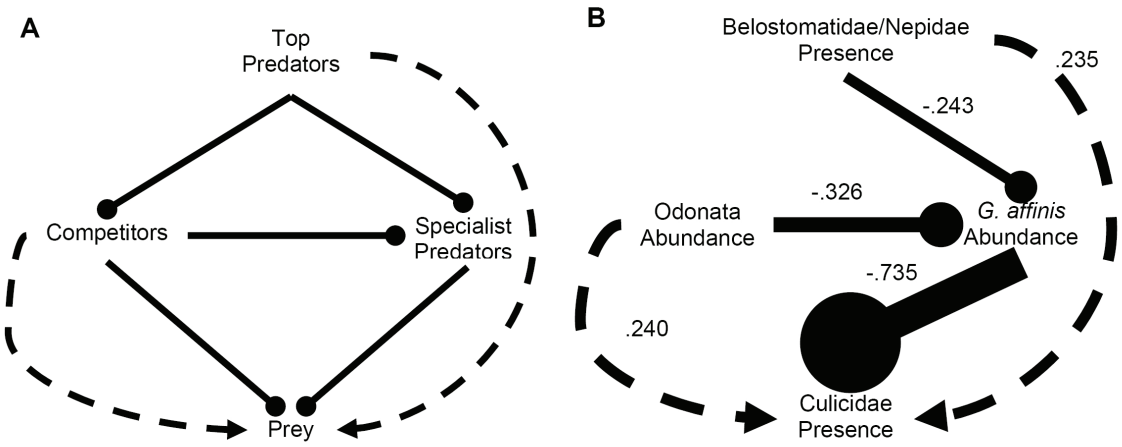

Figure 3. (A) Proposed model depicting expected direct effects (solid lines) and indirect effects (dashed lines) in a three-tiered food web with both specialist predators and other competitors present. The indirect effects (curved lines) of competitors and top predators on prey are expected to be positive due to direct suppression of specialist predators. Lines ending in arrows indicate a positive effect while those ending in circles indicate a negative effect. (B) Path diagram depicting observed direct (straight line) and indirect effects (curved line) of predators on the presence of mosquitoes in flooded swimming pools. Relationships that are statistically significant ($P<0.05$) are represented with solid lines. Widths of lines are proportional to the magnitude of effect with standardized coefficients next to each line. For this model, Chi-square = 0.152 with 1 degree of freedom resulting in a P-value of 0.696.

Orleans Parish during Spring 2006. Given the mitigating effect that top predators and competitors had on mosquito presence via suppression of *G. affinis*, these pools will need to be periodically reevaluated to determine the need for additional control measures.

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Bats: Important Reservoir Hosts of Emerging Viruses

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INTRODUCTION

The remarkable mammals known as "bats" and "flying foxes" (order Chiroptera ["hand wing"]) may be the most abundant, diverse, and geographically dispersed vertebrates (Table 1). Although a great deal is known about them, detailed information is needed to explain the astonishing variations of their anatomy, their lifestyles, their roles in ecosystems ecology, and their importance as reservoir hosts of viruses of proven or potential significance for human and veterinary health.

Bats fly with wings which range in span from 130 mm to 2 m. Bats of various species feed on insects, mammals, fish, blood, fruit, and pollen. Bats of most species echolocate to navigate and to find prey. Bats are found on all continents except Antarctica. Bats also are being increasingly recognized as reservoir hosts for viruses which can cross species barriers (i.e., "spill over") to infect humans and other domestic and wild mammals. Nonetheless, studies of the natural histories of bats and their importance as reservoir hosts of zoonotic viruses largely have been underappreciated and underfunded, except for their role in maintaining and transmitting rabies virus. Irrespective of the negative public perception of bats, they are critical elements of all terrestrial biotic communities. They help control insects, reseed cut forests, and pollinate plants that provide food for humans and other species, and their guano is used as a fertilizer and for manufacturing soaps, gasohol, and antibiotics (21, 69, 83). Bat echolocation and signal processing have provided models for sonar systems (112, 130).

Myths and misunderstandings about the roles of bats in ecosystems and their danger to other species as hosts of rabies virus have led to efforts to extirpate bat populations, with serious consequent effects on insect control and crop production, without coincidental reduction in the already low incidence of rabies virus transmission by bats (93).

This paper summarizes what is known about viruses isolated from bats. Although there is serologic evidence for infection of bats with many viruses (see, for example, references 82 and 101), we will focus here only on the 66 viruses that have been

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TABLE 1. Species of bats (order Chiroptera), by family and genus

Family and subfamily	No. of genera	No. of species
Megachiroptera, Pteropodidae	42	186
Microchiroptera		
Craseonycteridae	1	1
Emballonuridae	13	51
Furipteridae	2	2
Hipposideridae	9	81
Megadermatidae	4	5
Molossidae	16	100
Mormoopidae	2	10
Mystacinidae	1	2
Myzopodidae	1	1
Natalidae	3	8
Noctilionidae	1	2
Nycteridae	1	16
Phyllostomidae	56	160
Rhinolophidae	1	77
Rhinopomatidae	1	4
Thyropteridae	1	3
Vespertilionidae	47	407

isolated from or detected in bat tissues (Table 2) and the roles of bats in maintaining and transmitting viruses. Some of these bat-borne viruses can cause diseases of humans and other animals. The roles played by bats in the maintenance and transmission of viruses require consideration of the unique characteristics that distinguish bats from all other mammals. Examples are drawn from the extensive literature on rabies virus in bats, as well as from recent data on the roles of bats in the natural cycles of other viruses.

Evolution and Phylogeny of Bats

Whereas other mammals, such as certain species of rodents (order Rodentia) and carnivores (order Carnivora), may possess traits in common with species of bats, such as the ability to hibernate, no group of mammals shares the full suite of attributes that make bats unique. Of the more than 4,600 recognized species of mammals, 925 (about 20%) are bats (147). Bats are grouped into two suborders: Megachiroptera, containing a single family, Pteropodidae (42 genera, comprising 166 species), and Microchiroptera, containing 16 bat families (135 genera, comprising 759 species) (Table 1) (138).

Bats evolved early and have changed relatively little in comparison with mammals of other taxa (69). Although the fossil record of bat evolution is incomplete (77), a recent analysis of 17 nuclear genes dated the origin of chiropterans to the Eocene period (52 to 50 million years ago), coincident with a significant rise in global temperature (147). Three major microchiropteran lineages were traced to Laurasia and a fourth to Gondwana (147). The correspondingly ancient origins deduced for certain zoonotic viruses maintained in bats, such as the henipaviruses (60) and lyssaviruses (10), suggest a long history of cospeciation. Viruses that evolved with bats may have used for replication cellular receptors and biochemical pathways which are conserved in mammals that evolved later and which underwent radiation in later geological periods. If so, these conserved cellular receptors and pathways could enhance the capacity for transmission of bat-associated viruses to other mammals.

Ability To Fly

Bats are unique among mammals in their ability to fly. Bats fly daily in pursuit of food, and bats of many species fly long distances during seasonal migrations (62). For example, bats of *Myotis* spp. may travel 200 to 400 miles from their winter hibernation sites (reviewed in reference 62), and Mexican free-tailed bats (*Tadarida brasiliensis mexicana*) migrate at least 800 miles between their summer caves in Texas and New Mexico and their overwintering sites in Mexico (36) and are otherwise very widely distributed. In France, rabies virus infections have been associated with the migratory routes of Nathusius' pipistrelle (*Pipistrellus nathusii*) bats (20). Silver-haired bats (*Lasionycteris noctivagans*) seasonally range from Alaska, across Canada, and south to Texas (13). Rabies virus variants associated with silver-haired bats and the Eastern pipistrelle (*Pipistrellus subflavus*) have been identified from numerous locations throughout the geographic range of these bats (106, 124), and the same variants have been identified as the cause of the majority of cases of indigenously acquired human rabies in the United States and Canada (127).

Different patterns of migration within the same species of bat, as occurs with relatively solitary species, such as the silver-haired bat (69), and colonial cave-dwelling species, such as Mexican free-tailed bats (128), may permit exchange of novel viruses or virus variants between migrating and nonmigrating subpopulations of conspecifics or bats of other species. A Mexican free-tailed bat infected with a rabies virus variant normally associated with hoary bats (*Lasiurus cinereus*), suggests interspecies transmission (124). In the field, rabid bats of one species have been observed to be aggressive toward bats of other species (14). Moreover, Shankar et al. (136), in a study of the phylogenesis of divergence of rabies viruses from bats and terrestrial animals in Colorado, found that bats of different species had the same genotypic variants, indicating active interspecies transmission of rabies virus. They concluded that, at least in Colorado, animal rabies occurs principally in bats and that identification of bat-associated variants of rabies viruses in domestic cats, gray foxes (*Urocyon cinereoargenteus*), and striped skunks (*Mephitis mephitis*) demonstrates the importance of rabies virus spillover from bats to domestic and terrestrial wild vertebrates.

Torpor and Hibernation

An important trait of temperate bats of the families Vespertilionidae and Rhinolophidae is their ability to enter into daily torpor and seasonal hibernation to conserve energy during cool nights and winter months (89). The impact of torpor and hibernation on the pathogenesis and maintenance of viral infections in bats has not been studied extensively. However, viruses may overwinter in bats, and persistently infected bats may shed viruses, such as lyssaviruses (family *Rhabdoviridae*) or flaviviruses (family *Flaviviridae*) for extensive periods without evidence of disease (143). Virus isolation and antibody studies suggest that many viruses can cause persistent infections in bats (82).

When big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifigus*) were experimentally infected with Japanese encephalitis virus (JEV) and then subjected to temperatures

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TABLE 2. Viruses isolated from naturally-infected bats worldwide

Virus	Bat species (common name)[a]
Family *Rhabdoviridae*, genus *Lyssavirus*	
Rabies virus	Numerous bat species, essentially worldwide
Lagos bat virus	*Eidolon helvum* (African straw-colored fruit bat), *Micropteropus pusillus* (Peters' lesser epauletted fruit bat), *Epomops dobsonii* (Dobson's epauletted fruit bat), *Nycteris gambiensis* (Gambian slit-faced bat), *Epomophorus wahlbergi* (Wahlberg's epauletted fruit bat)
Duvenhage virus	*Miniopterus* sp., *Nyctalus noctula* (noctule), *Vespertilio murinus* (particolored bat), *Nycteris thebaica* (Egyptian slit-faced bat)
Australian bat lyssavirus	Megachiroptera (multiple *Pteropus* spp.), Microchiroptera sp. from Australia, *Saccolaimus flaviventris* (yellow-bellied pouched bat)
European bat lyssavirus 1	*Eptesicus serotinus* (common serotine), *Rousettus aegyptiacus* (Egyptian rousette)
European bat lyssavirus 2	*Myotis myotis* (mouse-eared myotis), *Myotis dasycneme* (pond myotis), *Myotis nattereri* (Natterer's myotis), *Miniopterus schreibersii* (Schreibers' long-fingered bat), *Rhinolophus ferrumequinum* (greater horseshoe bat), *Myotis daubentonii* (Daubenton's myotis)
Aravan virus	*Myotis blythii* (lesser mouse-eared myotis)
Khujand virus	*Myotis mystacinus* (whiskered myotis)
Irkut virus	*Murina leucogaster* (greater tube-nosed bat)
West Caucasian bat virus	*Miniopterus schreibersii* (Schreibers' long-fingered bat)
Family *Rhabdoviridae*, genus unassigned	
Gossas virus	*Tadarida* sp.
Kern Canyon virus	*Myotis yumanensis* (Yuma myotis)
Mount Elgon bat virus	*Rhinolophus eloquens* (eloquent horseshoe bat)
Oita 296 virus	*Rhinolophus cornutus* (little Japanese horseshoe bat)
Family *Orthomyxoviridae*, genus *Influenzavirus A*, influenza A virus	*Nyctalus noctula* (noctule)
Family *Paramyxoviridae*, genus *Henipavirus*	
Hendra virus	*Pteropus alecto* (black flying fox), *Pteropus poliocephalus* (gray-headed flying fox), *Pteropus scapulatus* (little red flying fox), *Pteropus conspicillatus* (spectacled flying fox)
Nipah virus	*Pteropus hypomelanus* (variable flying fox), *Pteropus vampyrus* (large flying fox), *Pteropus lylei* (Lyle's flying fox)
Family *Paramyxoviridae*, genus *Rubulavirus*	
Mapuera virus	*Sturnira lilium* (yellow epauletted bat)
Menangle virus	*Pteropus poliocephalus* (gray-headed flying fox)
Tioman virus	*Pteropus hypomelanus* (variable flying fox)
Family *Paramyxoviridae*, genus undetermined, a parainfluenzavirus	*Rousettus leschenaultia* (Leschenault's rousette)
Family *Coronaviridae*, SARS coronavirus	*Rhinolophus sinicus* (Chinese horseshoe bat), *Rhinolophus pearsonii* (Pearson's horseshoe bat), *Rhinolophus macrotis* (big-eared horseshoe bat), *Rhinolophus ferrumequinum* (greater horseshoe bat)
Family *Togaviridae*, genus *Alphavirus*	
Chikungunya virus[b]	*Scotophilus* sp., *Rousettus aegyptiacus* (Egyptian rousette), *Hipposideros caffer* (Sundevall's leaf-nosed bat), *Chaerephon pumilus* (little free-tailed bat)
Sindbis virus	Rhinolophidae sp., Hipposideridae sp.
Venezuelan equine encephalitis virus	*Desmodus rotundus* (vampire bat), *Uroderma bilobatum* (tent-making bat), *Artibeus phaeotis* (pygmy fruit-eating bat)
Family *Flaviviridae*, genus *Flavivirus*	
Bukalasa bat virus	*Chaerephon pumilus* (little free-tailed bat), *Tadarida condylura* (Angola free-tailed bat)
Carey Island virus	*Cynopterus brachiotis* (lesser short-nosed fruit bat), *Macroglossus minimus* (lesser long-tongued fruit bat)
Central European encephalitis virus	Unidentified bat
Dakar bat virus	*Chaerephon pumilus* (little free-tailed bat), *Taphozous perforatus* (Egyptian tomb bat), *Scotophilus* sp., *Mops condylurus* (Angola free-tailed bat)
Entebbe bat virus	*Chaerephon pumilus* (little free-tailed bat), *Mops condylurus* (Angola free-tailed bat)
Japanese encephalitis virus	*Hipposideros armiger terasensis* (great roundleaf bat; also known as Formosan leaf-nosed bat), *Miniopterus schreibersii* (Schreibers' long-fingered bat), *Rhinolophus cornutus* (little Japanese horseshoe bat)
Jugra virus	*Cynopterus brachiotis* (lesser short-nosed fruit bat)
Kyasanur Forest disease virus	*Rhinolophus rouxi* (rufous horseshoe bat), *Cynopterus sphinx* (greater short-nosed fruit bat)
Montana myotis leucoencephalitis virus	*Myotis lucifugus* (little brown bat)
Phnom-Penh bat virus	*Eonycteris spelaea* (lesser dawn bat), *Cynopterus brachyotis* (lesser short-nosed fruit bat)

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TABLE 2—*Continued*

Virus	Bat species (common name)[a]
Rio Bravo virus	*Tadarida brasiliensis mexicana* (Mexican free-tailed bat), *Eptesicus fuscus* (big brown bat)
St. Louis encephalitis virus	*Tadarida brasiliensis mexicana* (Mexican free-tailed bat)
Saboya virus	*Nycteris gambiensis* (Gambian slit-faced bat)
Sokuluk virus	*Vespertilio pipistrellus* (probably *Pipistrellus pipistrellus*; common pipistrelle)
Tamana bat virus	*Pteronotus parnellii* (Parnell's mustached bat)
Uganda S virus	*Rousettus* sp., *Tadarida* sp.
Yokose virus	Unidentified bat
Family *Bunyaviridae*, genus *Bunyavirus*	
Catu virus	*Molossus obscurus* (possibly *Molossus currentium*; Thomas' mastiff bat)
Guama virus	Unidentified bat
Nepuyo virus	*Artibeus jamaicensis* (Jamaican fruit-eating bat), *A. lituratus* (great fruit-eating bat)
Family *Bunyaviridae*, genus *Hantavirus*,	
Hantaan virus	*Eptesicus serotinus* (common serotine), *Rhinolophus ferrumequinum* (greater horseshoe bat)
Family *Bunyaviridae*, genus *Phlebovirus*	
Rift Valley fever virus	*Micropteropus pusillus* (Peters' dwarf epauletted fruit bat), *Hipposideros abae* (Aba leaf-nosed bat), *Miniopterus schreibersii* (Schreibers' long-fingered bat), *Hipposideros caffer* (Sundevall's leaf-nosed bat), *Epomops franqueti* (Franquet's epauletted bat), *Glauconycteris argentata* (common butterfly bat)
Toscana virus	*Pipistrellus kuhlii* (Kuhl's pipistrelle)
Family *Bunyaviridae*, genus unassigned	
Kaeng Khoi virus	*Chaerephon plicatus* (wrinkle-lipped free-tailed bat)
Bangui virus	*Scotophilus* sp., *Pipistrellus* sp., *Tadarida* sp.
Family *Reoviridae*, genus *Orbivirus*	
Ife virus	*Eidolon helvum* (straw-colored fruit bat)
Japanaut virus	*Syconycteris australis* (southern blossom bat)
Fomede virus	*Nycteris nana* (dwarf slit-faced bat), *Nycteris gambiensis* (Gambian slit-faced bat)
Family *Reoviridae*, genus *Orthoreovirus*	
Nelson Bay virus	*Pteropus poliocephalus* (gray-headed flying fox)
Pulau virus	*Pteropus hypomelanus* (variable flying fox)
Broome virus	*Pteropus alecto* (black flying fox)
Family *Arenaviridae*, Tacaribe virus	*Artibeus lituratus* (great fruit-eating bat), *A. jamaicensis* (Jamaican fruit-eating bat)
Family *Herpesviridae*, genus unassigned	
Agua Preta virus	*Carollia subrufa* (gray short-tailed bat)
A cytomegalovirus	*Myotis lucifugus* (little brown bat)
Parixa virus	*Lonchophylla thomasi* (Thomas' nectar bat)
Family *Picornaviridae*, genus undetermined,	
Juruaca virus	Unidentified bat
Unclassified	
Issyk-kul (Keterah virus)[c]	*Nyctalus noctula* (noctule), *Eptesicus serotinus* (common serotine), *Pipistrellus pipistrellus* (common pipistrelle), *Myotis blythii* (lesser mouse-eared myotis), *Rhinolophus ferrumequinum* (greater horseshoe bat), *Scotophilus kuhlii* (lesser Asiatic yellow house bat), *Cynopterus brachyotis* (lesser short-nosed fruit bat), *Eonycteris spelaea* (lesser dawn bat), *Chaerephon plicatus* (wrinkle-lipped free-tailed bat), *Hipposideros diadema* (diadem leaf-nosed bat), *Taphozous melanopogon* (black-bearded tomb bat), *Rhinolophus lepidus* (Blyth's horseshoe bat), *Rhinolophus horsfeldi* (possibly *Megaderma spasma*, lesser false vampire bat)
Mojui dos Campos virus	Unidentified bat
Yogue virus	*Rousettus aegyptiacus* (Egyptian rousette)
Kasokero virus	*Rousettus aegyptiacus* (Egyptian rousette)

[a] Species names and common names are given according to N. B. Simmons (138) and other sources.
[b] Arthropod-borne viruses (arboviruses) isolated from or detected in bats likely were transmitted to them by arthropods, whether from another individual of that bat species (reservoir host) or from another vertebrate reservoir host. With few exceptions, e.g., rabies virus, relatively little is known about the natural history of these viruses or about non-arthropod-transmitted viruses of bats.
[c] Issyk-Kul and Keterah viruses may be synonyms.

likely to be encountered during hibernation (8 to 24°C), individuals maintained viremias for 95 to 108 days (143). Virus titers in the blood of bats maintained at 24°C were equal to peak viral titers at temperatures at which the bats were active. Perhaps cold temperatures suppress immune responses that might otherwise control viremia. Bats transferred from 8°C to 24°C 9 weeks after inoculation with JEV had transient viremias followed by the rapid development of significant antiviral an-

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tibody titers. Nevertheless, the fact that infectious JEV was recovered from seropositive bats 15 weeks after the shift in temperature indicated that infection persisted (143). It is possible that neutralizing antibody has a shorter half-life in bats than in other mammals. Tick-borne encephalitis virus and other viruses have been isolated from bats with neutralizing antibody, and bats are susceptible to reinfection with tick-borne encephalitis viruses (82).

High titers of virus were obtained from brown fat of apparently healthy bats inoculated with rabies virus when the bats were kept at low temperatures (4, 142, 143). Vampire bats (*Desmodus rotundus*) that survive challenge with rabies virus may excrete virus in their saliva (1). Rabies virus was isolated from big brown bats that were captured to establish colonies and then died in the first month of captivity (135). Antiviral antibodies were detected in sera of several apparently healthy bats born in the new colony, suggesting past or subclinical rabies virus infection (135). Mexican free-tailed bats may transmit rabies virus transplacentally, as evidenced by the fact that infectious virus was isolated from cell lines established from fetal tissues of these bats (141). Studies of Mexican free-tailed bats roosting at a colony in Austin, Texas, identified rabies virus in about 70% of several hundred downed, dead, or dying bats, which represented a relatively small proportion of the estimated 600,000 bats in that colony. Over the study's 2-year duration, about 45% of apparently healthy bats from this roost were found to have neutralizing antibody to rabies virus, suggesting acquired immunity following prior exposure (101; C. Rupprecht [U.S. Centers for Disease Control and Prevention, Atlanta, Ga.], personal communication, 2006). Because only one or another of many methods usually is applied in studies of rabies virus in bats, we do not know the proportion of bats having both viral RNA in their tissues and antibody to rabies virus.

Temperate and tropical bats of the family Molossidae appear to be transitional between true hibernating bats and tropical bats that have limited ability to enter torpor. For example, the Western bonneted bat (*Eumops perotis*) enters a period of daily torpor during the winter that is similar to the daily hibernation or torpor that occurs in temperate zone bats during the summer (89).

Long Life Span

The extreme longevity of bats, together with the possibility that they might develop persistent infections with certain viruses, may help maintain the viruses and transmit them to other vertebrates. Many species of small temperate bats of the suborder Microchiroptera have life spans that exceed 25 years, with the greatest longevity, of 35 years, documented for a little brown bat. (On average, little brown bats weigh about 7 g.) This extreme longevity in a small mammal places bats well outside the traditional regression line for mammals that relates the life expectancy (9) to the ratio of metabolic rate to body weight (see reference 44, Fig. 45).

If bats routinely become persistently infected by certain viruses, and infectivity lasts for months or possibly years, the impact on the basic reproductive number of infection (R_0) would be significant. R_0 is the expected number of newly infected hosts that one infectious host will produce during its period of infectiousness in a large population of completely susceptible individuals (65). Since R_0 is the sum of the products of the average duration of infection, the average contact rate between infectious and susceptible individuals, and the probability of transmission per contact between an infectious and a susceptible individual, increased duration of infectiousness or increased prevalence of infection in a population can dramatically enhance the potential for secondary infections that emanate from a single infected individual. Persistent viral infections occurring among long-lived bats, coupled with their often gregarious roosting behavior, could greatly increase the potential for intra- and interspecies transmission of viruses.

Population Size and Roosting Behavior

The frequently great population densities of bats and their crowded roosting behavior increase the likelihood of intra- and interspecies transmission of viral infections. Bats are the most abundant of mammals, and except for humans and perhaps rodents, they are the most widely distributed land mammals (154). Certain species of bats, such as Mexican free-tailed bats, are highly gregarious and roost in southwestern caves of the United States, such as Carlsbad Caverns and Frio Cave, in densely packed aggregates of approximately 300 bats per ft^2 (37), in populations comprising several million individuals (37, 94). Under these conditions the only example of airborne rabies virus transmission was documented, either in droplets of excreta or by small particle aerosol (38, 155).

Bat Population Structure

The demographic and spatial structuring of bat populations is sufficiently variable to offer opportunities for viruses that cause both acute and persistent infections to be maintained. The potential for migratory and nonmigratory populations to serve as a mixing vessel for viruses has already been mentioned. Additionally, within given regions, bat populations may be panmictic or may exist as metapopulations, offering the potential for seasonal virus transmission and annual outbreaks of viral diseases as well as the potential for periodic outbreaks among spatially discrete populations.

Colonial microchiropterans (such as Schreibers' long-fingered bat, *Miniopterus schreibersii*, and Mexican free-tailed bats) typically exist in panmictic populations of hundreds of thousands or millions of individuals and produce an annual birth pulse (37). In theory, such large bat populations could sustain acute viral infections that produce permanent sterilizing immunity in affected individuals in a manner akin to that of measles morbillivirus, which persists to cause annual outbreaks only when human communities exceed 250,000 to 500,000 (16). The persistence of measles virus within demographically heterogeneous human populations, whereby different communities are affected in different years, may give rise to viral persistence in spatially discrete "patches," in which infection dies out sequentially rather than simultaneously (17).

A different pattern of social structure is present among other colonial bats that have a metapopulation structure (consisting of periodically interacting, spatially discrete subpopulations). Flying foxes (*Pteropus* spp.) have such a structure. In this situation, the total number of individuals in the various subpopu-

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lations or "patches" must be sufficient to maintain virus circulation in the metapopulation over time, while immunity or death due to viral infection extinguishes transmission chains within individual subpopulations. Periodic outbreaks of viral infection and disease may then be expected among given subpopulations in a region, once the number of susceptible individuals has recovered through births or loss of immunity, such that the populations once again can support viral transmission with an R_0 of >1. Such periodic outbreaks of acute, even fatal viral disease are well documented for rabies virus among terrestrial carnivores (28) and may occur among vampire bats, as exemplified by the so-called "migration" of rabies virus in vampire bat populations in different regions with a 2- to 3-year cycle (19, 126). Preliminary modeling suggests that Hendra virus persists in Australian flying foxes in this way (H. E. Field, unpublished data).

Given that the phylogenetic distance of Hendra virus (and Nipah virus) from other viruses in the family *Paramyxoviridae* suggests that these are ancient viruses that likely have an evolutionary association with their flying fox hosts, it is both intuitive and biologically plausible that the maintenance of Hendra virus infection in flying foxes is based on the spatially heterogeneous population structure and nomadic nature of flying foxes.

Echolocation

Microchiropteran bats are, with rare exceptions among the Megachiroptera (69, 71), the only land mammals that emit sounds and then detect and characterize the time delay and signal properties of returning echoes for the purpose of navigation (echolocation). Although certain birds and several species of megachiropterans use primitive echolocation, the degree to which neural and muscular systems of bats have evolved to produce echolocation signals, protect the individual bat from its own potentially deafening emissions, and decipher the information contained in returning echoes is unique. However, acoustic imaging is energy-intensive, corresponding to an energy flux of as much as 6×10^{-6} J/m^2 per echolocation call (113). The intense, high-frequency echolocation signals, ranging between 80 and 110 dB at a distance of 1 m from the emitting bat, approximate the range between the noise level produced by a coffee grinder and that produced at a rock concert or by a jet plane at ramp (5, 113). Echolocation signals are produced by the larynx, are powered by the muscles of the abdominal wall of bats, and are emitted through the mouth or nostrils (113). Production of such loud sounds also could generate droplets or small-particle aerosols of oropharyngeal fluids, mucus, or saliva, enabling transmission of viruses between individuals in close proximity. The hypothesis that rabies virus could be expelled from the nostrils of echolocating bats was supported by the isolation of rabies virus from mucus obtained from naturally infected Mexican free-tailed bats (39).

Bat Immunology

Why can certain viruses infect and persist in apparently healthy bats yet be highly pathogenic for humans and other vertebrates? Because bats were among the earliest mammalian species to develop, it is possible that their innate and acquired immune responses have important qualitative or quantitative differences from those of the rodents and primates which have been studied extensively. Do bats have a different set point in their immune responses, one that results in control of the level of virus replication without clearance of infectious virus in order to prevent immunopathological responses in infected tissues? Are all of the innate immune mechanisms that are presumed to have preceded the development of acquired immune responses also functional in bats? Is there affinity maturation of antibodies in bats? What are the properties of cell-mediated immune responses in bats? Significant differences in immune responses to viral infection likely will be found among the very large number and diversity of bat species, and it is unlikely that immunological reagents will be reactive across all bat species.

Very little is known about bat immune systems, although several studies suggest that immune responses of bats have some similarities with those of mammals that evolved after bats. For example, immunoglobulin G (IgG), IgA, and IgM have been purified from sera of great fruit-eating bats (*Artibeus lituratus*) (96). Macrophages, B- and T-lymphocyte-like cells, and cells expressing surface Ig were identified in the bone marrow of Indian flying foxes (*Pteropus giganteus*), indicating that lymphoid development is generally similar in bats and other mammals (26, 131). Presumably in bats, as with other mammals, the generation of high-titer IgG requires two events mediated by helper T cells: class switching and affinity maturation.

Serological assays that detect IgG antibodies to Hendra virus, severe acute respiratory syndrome coronavirus (SARS-CoV)-like viruses, and Ebola viruses in bats (66, 84, 85) indicate that some virus-specific adaptive T- and B-cell responses occur despite persistent virus infection. Further studies will require development of cell culture-based assays for examining lymphocyte proliferation, antibody synthesis, cytokine synthesis, and a host of other immunologic functions in bats.

VIRUSES FOUND IN BATS

Table 2 lists the large number of viruses that have been isolated from or detected in bats, but most of these viruses have not been shown to be transmitted from bats to other animals or to cause human disease. Transmission from bats of viruses causing highly pathogenic disease has been demonstrated for rabies virus and related lyssaviruses, Nipah and Hendra viruses, and inferred for SARS-CoV-like virus of bats. The relationships of these viruses to their bat hosts and to zoonotic human diseases is described below. Other viruses in Table 2, such as certain alphaviruses, flaviviruses, and bunyaviruses, may infect bats via arthropods, but it is not clear whether bats are important reservoir hosts for these viruses. Clearly, a great deal of additional research is needed to document the roles of bats of different species in the natural history of the many viruses for which these remarkable animals can serve as hosts.

Rabies Virus

It would be impossible here to summarize the scientific literature with regard to rabies and rabies virus. Therefore, we

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will merely summarize what we believe is relevant to this review. Descriptions of a disease consistent with rabies date from 4,000 years ago. The Eshnunna code invoked penalties for knowingly allowing a "mad" dog to bite a human (12). In the first century of this era, Celsus warned of fatal bites from animals and suggested that such bites may contain venom (i.e., "virus"). However, it was not until the late 19th century that rabies virus was studied methodically. Louis Pasteur amplified the virus in rabbit spinal cord and prepared and administered a vaccine for postexposure prophylaxis. Those classical studies laid the foundations for virology and immunology.

Rabies virus (family *Rhabdoviridae*, genus *Lyssavirus*, serotype 1/genotype 1) is transmitted between mammals, including bats, primarily through the bite inoculation of rabies virus present in the saliva of infected individuals (95). The dual characters of transmitting rabies virus and being hematophagous (i.e., vampire bats) have cast a shadow on bats. Bats of three species (*Diphylla ecaudata* [hairy-legged vampire bat], *Diaemus youngi* (white-winged vampire bat), and *Desmodus rotundus* [vampire bat]) are known vampires and have been found to be involved in transmission of rabies virus, although available evidence indicates that only the latter is important in this regard (149).

Globally, a vanishingly small proportion of the approximately 55,000 annual human deaths caused by rabies virus are caused by variants of virus associated with bats (81). Although most cases of indigenously acquired human rabies in the United States are caused by bat-associated variants of rabies virus, the average of 1 or 2 cases per year over the past 2 decades indicates the rarity of these events (101). In the United States, most rabies victims do not recall having been bitten by a bat, which may be due to the small size of the biting animal or to unusual circumstances leading to the bite (127).

Recent evidence suggests that all rabies virus variants that affect terrestrial carnivores originated from cross-species transmission of bat-associated variants of rabies virus (10). A molecular clock model based on genetic divergence of rabies virus variants in bats of different species suggests that in North America the divergence of extant bat-associated rabies viruses from a common ancestor occurred about 1651 to 1660 C.E. The bat rabies virus variants found in Latin America in common vampire bats (*Desmodus rotundus*) and in free-tailed bats (genus *Tadarida*, family Mollosidae) are closest to the earliest common ancestor. Adaptation of rabies virus variants occurred earlier and more rapidly in bats of colonial genera (genera *Eptesicus* and *Myotis*) than in bats of more solitary genera (*Lasionycteris*, *Pipistrellus*, and *Lasiuris*) (74).

Bat variants of rabies virus sporadically spill over to infect mammals other than humans (97). Sustained transmission of bat variants of rabies virus within populations of red foxes on Prince Edward Island and striped skunks in Arizona (40, 45) proceeded until natural extinction or control by vaccination.

Lyssaviruses Related to Rabies Virus

Rabies virus is related to other lyssaviruses from bats, rodents, and arthropods (137). There are seven lyssavirus genotypes and an additional four novel genotypes recently recovered from bats in Eurasia (Table 2), which probably will be included in this genus (67, 151). Some of these viruses, most notably Australian bat lyssavirus (ABLV) (140), can cause a fatal human illness indistinguishable from classic rabies (68, 129), but other lyssaviruses are not known to cause disease in vertebrates. The diagnosis of rabies in humans and animals traditionally was restricted to the acute fatal encephalomyelitis caused by rabies virus serotype 1/genotype 1, but now the disease "rabies" includes any of the fatal illnesses caused by any lyssavirus (67).

Details of the maintenance cycles for lyssaviruses other than rabies virus, such as Duvenhage, Lagos bat, and Mokola viruses (Table 2), are unclear (111). However, as with rabies virus, their perpetuation is assumed to involve bite transmission, primarily involving conspecifics of the reservoir host species, with occasional spillover to other susceptible vertebrates. Individuals of other species have been sporadically found to be infected by these rarely identified lyssaviruses, including a human with Duvenhage virus (100, 144), domestic cats and a dog with Lagos bat virus (54, 80, 98), and humans, domestic cats, and dogs with Mokola virus (15, 46, 47, 53, 110).

In May 1996, a lyssavirus was isolated from tissues of a black flying fox (*Pteropus alecto*) with signs of encephalitis found near Ballina, New South Wales, Australia (55). Six months later, a bat handler from Rockhampton, Queensland, Australia, developed numbness and weakness in her arm and later died from encephalitis. She had been infected with what is now known as ABLV. In 1998, a woman from Mackay (Queensland, Australia) was diagnosed with ABLV infection at her death, 2 years after having been bitten by a sick bat (68). Protection trials with mice conducted at the U.S. Centers for Disease Control and Prevention, Atlanta, Ga., indicated that a rabies human diploid cell vaccine might be useful for prophylaxis against this virus (90). Recent serologic evidence suggests that this virus also is present in bats in Thailand (88). Because of the colonial nature of many bats, it is likely that this virus may be found wherever the host bats are found.

Henipaviruses

In 1994 an outbreak of an acute respiratory illness occurred in a human and 14 horses in Hendra, a suburb of Brisbane, Australia. Twenty-one horses and two humans (the trainer and a stable hand) were infected (109). Four additional outbreaks, in 1994, 1999, and 2004, infected five horses and two humans, killing all but one human (49, 72, 116, 123, 133).

A virus (family *Paramyxoviridae*, genus *Henipavirus* [named after Hendra and Nipah viruses]) was shown to be the etiologic agent of this disease (109). The natural hosts and probable reservoirs of Hendra virus are fruit bats ("flying foxes") of the genus *Pteropus*, including the black flying fox (*Pteropus alecto*), gray-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spectacled flying fox (*P. conspicillatus*) (50). Little is known about the dynamics of infection in flying foxes and how Hendra virus infection is maintained in them.

Field (50) proposed three alternative models for the maintenance of infection: (i) infection is enzootic in all species throughout their distribution; (ii) infection is enzootic in a particular species with a periodic epizootic pattern in the other species; or (iii) infection is periodically epizootic in all these species, persisting in a spatial or temporal mosaic across their distribution. He contends that the apparent pattern of known

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"spillovers" from flying foxes to horses fits better with either of the two latter hypotheses. That is, a periodic outbreak in a local population of flying foxes results in an increased probability of spillover to horses in a specific locality during a limited time period. An outbreak of Hendra virus infection in a local population of flying foxes may depend on attainment of a threshold number of susceptible flying foxes in the population and introduction of the virus into the population from a nomadic individual or group. These concepts are well studied for related morbilliviruses (17, 146). A situation analogous to the circumstances being proposed for spillover of Hendra virus to horses has been described for rabies virus spillover to domestic cats. In the eastern United States, there is a strong association between the local temporal dynamics of rabies epizootics within a reservoir host species, in this case the raccoon (*Procyon lotor*), which serves as the regional reservoir host for a specific variant of rabies virus, and an increase in the risk of rabies spillover to domestic cats (59).

Nipah virus, a paramyxovirus related to Hendra virus, was first isolated in 1999 from pigs and adult human males affected by fever and encephalitis, some with respiratory illness, during a major outbreak in peninsular Malaysia and then in Singapore (23, 24, 31). Of 265 reported human cases, 105 were fatal. Direct contact with infected pigs was identified as the predominant mode of human infection (33, 57). Most of the humans affected in the Malaysian outbreak had a history of direct contact with live pigs, and most were adult male Chinese pig farmers (31, 117). More than 1 million pigs were culled to contain the outbreak. With the knowledge that *Pteropus* species bats were the likely reservoir of the closely related Hendra virus in Australia, Malaysian bats were prioritized for surveillance. Like most other countries in Southeast Asia, Malaysia has a great diversity of bat species, including 13 species of Megachiroptera and 60 species of Microchiroptera (99). The large flying fox (*Pteropus vampyrus*) and the variable flying fox (*P. hypomelanus*) were found to be natural reservoir hosts for Nipah virus (34, 76).

Since 2001, sporadic outbreaks of Nipah virus-associated disease in humans have been identified in Bangladesh (6, 7, 8, 73). Although many characteristics of these outbreaks were similar to those of the Malaysian outbreak, including delayed recognition, a primary presentation with fever and central nervous system signs, and a high case fatality rate, in Bangladesh the human cases were not associated with disease in pigs, and there was some evidence suggesting human-to-human transmission (73). Serologic surveys of domestic and wild animals undertaken after the 2001 and 2003 outbreaks in Bangladesh provided evidence of Nipah virus infection only in Indian flying foxes (6, 73). Concurrent serologic surveillance of Indian flying foxes in India in 2003 found that 54% had neutralizing antibodies to Nipah virus (J. H. Epstein et al., personal communication, 2006), suggesting that Nipah virus or a closely related virus was widespread across the range of Indian flying foxes. Chadha et al. (25) recently reported the occurrence of Nipah virus infections in humans in India in 2001. Neutralizing antibodies to Nipah virus were found in large flying foxes in Indonesia (134) and Cambodia (114), and Nipah virus was isolated from Lyle's flying fox (*Pteropus lylei*) in Cambodia (121). Thus, the henipaviruses likely occur across the entire global distribution of pteropid bats (66).

Available evidence suggests that Hendra and Nipah viruses are ancient viruses that have long circulated in their natural hosts, flying foxes (60). What precipitated the apparent recent emergence of these viruses? Can we identify environmental factors that altered flying fox ecology and facilitated the movement of henipaviruses (and other bat-associated zoonotic agents) beyond their natural ecological niches? Disease emergence requires, in addition to the presence of an agent, an effective bridge from the natural host to a susceptible spillover host. Such bridges may be caused by changes to the agent, the host, or the environment. Data on fruit bats of many species suggest that populations are in decline throughout their range, primarily as a result of habitat loss and hunting. In Australia, fruit bat roosting sites recently have been increasingly redistributed to urban areas (64). A scenario emerges of flying fox populations under stress, altered foraging and behavioral patterns, and virus niche expansion, all leading to closer proximity to humans and livestock. This certainly was the case with Nipah virus emergence (35). Chong et al. (30) suggested that the risk of humans contracting Nipah virus infection from bats is low. Once Nipah virus escapes its natural cycle, its epidemiologic characteristics are quite a different story.

Menangle and Tioman Viruses

Menangle virus (family *Paramyxoviridae*, genus *Rubulavirus*) was isolated in 1997 from stillborn piglets at a large commercial piggery near Menangle in Australia (118); the bat colony and the piggery had coexisted for 29 years before the incident. There were large numbers of within-litter fetal deaths at a variety of gestational ages. Most sows carried their litters to term, but abortions occasionally occurred. Affected litters included mummified, autolyzing, fresh stillborn, and live piglets. Teratogenic defects frequently seen included arthrogryposis, brachygnathia, and kyphosis. Internally, part or all of the brain and spinal cord was absent in most piglets, and there was malacia and nonsuppurative inflammation of the brains and spinal cords of some. Nonsuppurative myocarditis and hepatitis also were present in some piglets (118).

Two of 250 humans in contact with the infected pigs had high titers of antibodies to the new virus, and both reported a febrile illness with a measles-like rash, but neither had direct exposure to flying foxes (27). Individual bats living in a large, mixed colony of gray-headed flying foxes and little red flying foxes seasonally, and roosting within 200 m of the affected piggery, had neutralizing antibodies (118), as did flying foxes of other species from other colonies thousands of kilometers distant and previous to the outbreak at Menangle (Field, unpublished); other species in the vicinity of the affected piggery were seronegative. Although attempts to isolate virus from flying foxes were unsuccessful, paramyxovirus-like virions labeled with antibody to Menangle virus from a convalescent sow were seen by electron microscopy in flying fox feces collected beneath the roost near the piggery.

Tioman virus, a rubulavirus distinct from Menangle virus, has been isolated from variable flying foxes in Malaysia. Little is known about the host range or pathogenesis of this newly recognized paramyxovirus (32).

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SARS-CoV-Like Viruses of Bats

In 2002, a previously unrecognized coronavirus (family *Coronaviridae*) was found to cause a new, severe acute respiratory syndrome in humans (92, 125). This virus, named SARS-CoV, is a distant relative of the group 2 coronaviruses that infect rodents, cattle, dogs, pigs, and humans and has been assigned to group 2b (58). It is distinct from two other coronaviruses recently identified in bats in southern China (84, 119).

Epidemiologic studies showed that the earliest cases of SARS were associated with the wildlife meat industry. A survey of wildlife in a Shenzhen market recovered SARS-CoV-like viruses from masked palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) and detected antibodies to the SARS-CoV-like virus in a hog badger (*Arctonyx collaris*) (63). Interestingly, the epidemiology of the outbreak in animals in the wildlife meat market resembled that of shipping fever, a viral syndrome that occurs when animals from different farms are comingled under crowded, stressful conditions. Under such circumstances, immune responses to persistent virus infections are reduced, virus shedding is increased, and susceptible animals become infected and shed virus. In the marketplace where SARS-CoVs were detected, viral RNA from some animals that were seronegative was detected by reverse transcription-PCR, suggesting acute infection, while other animals had antibodies to SARS-CoV but continued to shed virus, suggesting persistent infections (63). Although no pathology was associated with SARS-CoV in animals in this market, civets inoculated with human isolates of SARS-CoV had severe lung pathology (156). By sequencing many viral genomes from SARS patients, wild and farmed civets, and other animals, a dendrogram was generated that showed that the first human SARS coronaviruses were closely related to a contemporary virus from masked palm civets and that point mutations were selected and accumulated later, as the virus passed from human to human (139).

Extensive surveys of viruses in domestic animals, poultry, and wildlife were done by reverse transcription-PCR to identify the natural reservoir of SARS-CoV. Palm civets were found to be an incidental host rather than the principal host for SARS-CoV. Recently, several groups simultaneously identified bats from different locations in southern China as being infected with SARS-CoV-like viruses or having antibody to these newly recognized coronaviruses, including members of several species of Chinese horseshoe bats (suborder Microchiroptera, family Rhinolophidae, genus *Rhinolophus*) (Tables 1 and 2) (42, 84, 86). The prevalence of antibody to bat SARS-CoV in some species of Chinese horseshoe bats was as high as 84%. Pathology has not yet been associated with SARS-CoV infection of bats.

The genomes of SARS-CoV isolates recovered from civets and humans during the 2002-to-2003 outbreak of SARS lay phylogenetically within the broad group of SARS-CoV-like viruses of bats (86). These data show that the virus responsible for the 2002-to-2003 outbreak most likely originated from this group of bat-associated viruses. Antibody against SARS-CoV-like viruses of bats was also detected in Leschenault's rousette (*Rousettus leschenaultia*), a cave-dwelling megachiropteran, suggesting that fruit bats also may support infection with SARS-CoV-like viruses. Thus, the natural history of SARS-CoV appears to involve a previously unrecognized SARS-CoV-like virus of bats being transmitted in meat markets to amplifying hosts, including masked palm civets, raccoon dogs, and a hog badger, and then spilling over to infect humans in close contact with these intermediate hosts or their tissues. Subsequent human-to-human transmission of the virus was associated with adaptive mutations in the viral genome (139).

Ebola Viruses

Five viruses have been placed in the taxon *Filoviridae*. Four of them (Ebola Zaire virus, Ebola Sudan virus, Ebola Ivory Coast virus, and Ebola Reston virus) comprise the genus *Ebolavirus*; Marburg virus comprises the genus *Marburgvirus*. The natural reservoir hosts of these viruses have not yet been identified. However, Ebola virus RNA has been detected in terrestrial mammals in the Central African Republic (107). Experimental infections of the Angola free-tailed bat (*Mops condylurus*), little free-tailed bat (*Chaerephon pumilus*), and Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) with Ebola Zaire virus led to replication of virus in these bats (145). Recently, Ebola virus RNA was detected in liver and spleen tissues of three fruit bats: the hammer-headed fruit bat (*Hypsignathus monstrosus*), Franquet's epauletted bat (*Epomops franqueti*), and little collared fruit bat (*Myonycteris torquata*) (85). Ebola virus-specific immunoglobulin M antibody was detected in bats of the same species, but Ebola virus RNA was not detected in bats with antibody, and antibody was not detected in bats with Ebola virus RNA.

Detection of Ebola virus RNA in bats and rodents is a fascinating finding, as is detection of antibody. However, until and unless an Ebola virus (or Marburg virus) is isolated from a wild vertebrate, and experimental infections unambiguously demonstrate that the virus not only persists but is shed by that animal and that disease can be transmitted under controlled conditions, these findings will remain simply intriguing and promising. Monath has postulated that there may be an as-yet-undetected Ebola virus, one that is nonpathogenic but may give rise to pathogenic genotypes by mutation, and that the filoviruses may be arthropod or plant viruses (105).

IMPLICATIONS FOR FUTURE RESEARCH ON EMERGING VIRUSES OF BATS AND OTHER WILDLIFE

Emerging Viruses and Wildlife Surveillance

Scores of newly recognized viruses have emerged in recent decades, and elegant reviews have brought into focus the continuing importance of this phenomenon (91). It seems surprising, as though we are caught unawares, when a hitherto unrecognized disease and its causative virus are discovered. Recognition of the spillover of a zoonotic virus is precipitated by human, livestock, or wildlife deaths, with considerable medical, emotional, and economic miseries. We wonder how such a virus could have evaded detection, why it had not been seen to cause disease before, and whether it is a "new" virus. When new emerging zoonotic viral diseases appear, reviews and grant applications are written, explanations proffered, symposia or-

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ganized, and molecular and other specific diagnostic tools developed. Unfortunately, one important method to predict emergence of zoonotic diseases that has been overlooked repeatedly is the natural history survey, followed by targeted studies of species of interest identified through the survey. Survey research followed by targeted study has been used successfully to explore the epidemiology of reservoir host-zoonotic virus maintenance, as exemplified by studies on hantaviruses in the southwestern United States (102, 103). These studies have helped epidemiologists and public health officials make recommendations to reduce the risk of infection and to help forecast the location and severity of future outbreaks of hantavirus pulmonary syndrome (22, 51, 52, 56).

From about 1930 to about 1970, governments and private institutions emphasized sending people into the field to count, trap, measure, bleed, and test vertebrates and invertebrates for viruses. Although those surveys may not have provided answers directly and quickly, they did provide specimens for future analyses and questions to be addressed. Many "orphan" viruses without known connection to disease were discovered. Information regarding more than 500 viruses was collected by the American Committee on Arthropod-Borne Viruses and published in the *International Catalogue of Arboviruses Including Certain Other Viruses* (78). This invaluable but badly outdated compendium is slowly being replaced by Internet resources as a means of information dissemination. Supplementing and replacing this printed catalogue are numerous databases (e.g., ICTVdB, The Universal Virus Database of the International Committee on Taxonomy of Viruses, available at http://www.ncbi.nlm.nih.gov/ICTVdb/). Nevertheless, most of the current data are limited in scope and in imagination. What is needed is a survey of viruses of all vertebrates, invertebrates, plants, and other life forms. Although thoughts of complete surveys obviously are wishful thinking, some survey efforts are better than none.

Information about the natural history of most viruses in bats is limited. Regarding the conservation status of 914 bat species listed by Wilson (153), omitting those that are, or are suspected to be, extinct, 390 (42.7%) are considered not assessed (adequately), 297 are considered stable, 201 are considered vulnerable or potentially vulnerable, and 26 are considered endangered. Of the 390 species that have not been assessed adequately, 38 (9.7%) are of the family Pteropodidae, the Old World fruit bats, from which the most recent virus emergences have been observed. These 38 represent 59.4% of the 64 genera in this family, indicating that we know relatively little about the bats from which zoonotic viruses that cause human disease have recently emerged. To various degrees, this can be said about all bats, if not about all vertebrates.

Obviously, there is a need for comprehensive surveys of bats in every place where they occur, although conservation concerns must be addressed in the design of survey and sampling methods, given that bats of many species are protected. Nonkilling techniques involving bleeding or procuring of oropharyngeal and rectal swabs for PCR-based analyses, in addition to collection of recently dead individuals, have been used to determine viral infections and the prevalence of antibodies in bats (see, e.g., references 43, 114, and 115). We know very little about the 925 bat species that have been recognized, placed in a taxon, and largely ignored after that. How many unrecognized viruses do those bats harbor? Will any or all of these viruses eventually be shown to be human, livestock, or wildlife pathogens? What new viruses lurk in the other nearly 4,000 species of mammals and the thousands of species of other vertebrates, invertebrates, plants, and individuals of other kingdoms, phyla, and classes? In effect, without even partial predictive capacity, we are simply waiting for the next disastrous zoonotic virus outbreak to occur. Clearly, this is not an effective prevention or prediction philosophy.

However, there is no simple solution to this need. Perhaps part of the problem is lack of interest (ignorance?), and part is due to lack of funding (there is not enough money for everything). Surely in some countries, principally those from which emerging disease are emerging, there is a lack of infrastructure, manpower, and even national will; these are political questions. We suggest holding international symposia emphasizing the importance of both natural history surveys and of knowledge as not only predictive tools but also disease-prevention tools. Further emphasis on greater prioritization of such studies might be shown to be very cost-effective in the long run.

Virus Isolation and Characterization

Virus isolation techniques and PCR assays now are extremely sensitive and rapid. These methods could provide the opportunity to collect and store a massive amount of information to accompany bat sera and tissue specimens. This would provide us with at least some degree of intellectual preparedness and with reagents that could be used to develop rapid diagnostic assays for newly emerged viruses.

When a newly recognized virus is detected, virus identification is now done by PCR amplification of viral nucleic acid, and the resulting sequence data are compared with sequences in the genetic databases, such as GenBank (http://www.ncbi.nih.gov/GenBank/), to search for similarities with sequences of recognized viruses. In addition, viral proteins (antigens) can be expressed and used for serodiagnostic tests. If possible, the infectious virus is isolated, antigens are prepared for diagnosis, and experimental infections are conducted to study pathogenesis. Emerging viral diseases often are misdiagnosed. For example, when the Nipah virus infection was first reported in Malaysia in 1999, the diagnosis was "JEV infection," even though (i) all the human patients were adult males, (ii) most or all of those people had been vaccinated against JEV, (iii) pigs suffered fatal disease (pigs do not die when infected with JEV; they serve as amplifying hosts for that virus), and (iv) a virus isolated from patients with the disease appeared by electron microscopy to be a paramyxovirus, not a flavivirus, as JEV is. Only after it was realized that an intensive JEV vaccination campaign was not diminishing transmission of this new disease were other approaches initiated; by then, valuable time and many lives had been lost. That outbreak can serve as an example of our perpetual following of epidemic curves rather than predicting them, of our lack of early recognition of emerging diseases.

Diagnostic Limitations

At this time, diagnostic reagents or tests are available for all the viruses shown in Table 2. However, to detect previously

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unrecognized viruses, new reagents and approaches must be developed or existing techniques applied. Among the new reagents, a variety of nested primers useful for exploratory PCR might be formulated based on knowledge of sequences of recognized viruses within the order *Mononegavirales* (which includes *Bornaviridae*, *Rhabdoviridae*, *Filoviridae*, and *Paramyxoviridae* [120]). In addition, bat family-specific or genus-specific conjugates could be produced and applied for use in immunofluorescence assays or in enzyme-linked immunosorbent assays to identify antibodies in sera or blood samples, or antigens in tissue samples. Classical methods including hemagglutination-inhibition tests, which are broadly cross-reactive, also could be developed using inactivated antigens prepared from various recognized viruses. Virus isolation assays, while potentially quite hazardous, also can be applied if used with appropriate biocontainment. In this day of increasing emphasis on molecular genetic tools for detecting viral nucleic acids and for identifying nucleotide sequences rather than the viruses themselves, it is frequently overlooked that virus isolation provides us with a virus. With the virus itself, many areas of research and development can be addressed, including development of diagnostics, of animal disease models, and of vaccines. Emphasis, sometimes complete emphasis, on nucleotide sequence characterization rather than virus characterization has led us down a primrose path at the expense of having real viruses with which to work.

Studies on Immune Responses of Bats

To understand the innate and acquired immune responses of bats during acute and chronic virus infections, much additional research is needed. It will be necessary to develop bat cell culture-based assays and bat-specific reagents to examine lymphocyte proliferation, antibody and cytokine synthesis, cell-mediated immune responses, and a host of other immunologic functions in bats that are important reservoirs of emerging viruses. A major challenge in studying T-cell responses in bats is the apparent lack of inbred strains of bats. Such animals are needed for long-term T-cell studies because of the requirement for matched major histocompatibility complex molecules on T cells and antigen-presenting cells. Colonies of captive bats might carry zoonotic viruses that could be transmitted to humans, so research on the bats and their cells might require biological containment. In rodents, the growth factors required for in vitro expansion and maturation of bone marrow stem cells into competent antigen-presenting cells have been partially characterized, leading to development of cell culture assays for intermediate-term propagation of rodent T cells (41). Similar strategies likely can be employed for propagation of T cells from bats of various species.

Molecular genetics should be useful for analyzing bat immune responses. More than 4,000 protein-encoding sequences from chiropterans are in the National Center for Biotechnology Information databases (http://www.ncbi.nlm.nih.gov/), but of these only sequences for recombination activation genes 1 and 2 are immunologically relevant. Despite this limitation, it should be possible to develop assays for evaluating such responses in infected bats. Perhaps most tractable and meaningful for understanding these responses are analyses of cytokines and chemokines, especially in conjunction with cell culture assays. Capture enzyme-linked immunosorbent assays and flow cytometry-based assays for a number of cytokines and chemokines from conventional species have permitted an elegant dissection of immune responses in humans and rodents. However, development of monoclonal antibody pairs for cytokine detection assays requires substantial funding and effort. More recent developments employing molecular approaches, such as real-time PCR, cDNA arrays, and RNase protection assays, have accelerated development assays for cytokine and chemokine gene expression. These assays will require sequencing of bat orthologs, but considering that 11 assemblies of mammalian genomes are already available, it is likely that most genes from bats of most species could be cloned and sequenced using degenerate PCR primer sets, a strategy that has been used for other species (132). Once the relevant gene sequences are known for bats of a given species, real-time PCR assays could be developed. In conjunction with cell culture studies, it should be possible to characterize bat immune responses to challenge with viral antigens. Bacterial artificial chromosome libraries are available for the little brown bat and the greater horseshoe bat, a species closely related to the Chinese horseshoe bat (*Rhinolophus sinicus*), a host for SARS-CoV-like virus (84; http://bacpac.chori.org/libraries.php). These resources may be particularly valuable for rapidly identifying immune response or cytokine genes of interest by using human or mouse hybridization probes.

Immune Evasion and Virus Persistence

Viruses must evade the host immune response for a time sufficient to allow transmission to other susceptible hosts or to establish persistent infection. The strategies employed by viruses are numerous and target both the innate and adaptive phases of the immune response. Some commonly employed evasion strategies include virus-encoded immune-modulating cytokines, decoy soluble cytokine receptors, inhibitors of apoptosis and cellular signaling, inhibitors of antigen processing, and T-cell antagonists (2, 3, 18, 61, 70, 79, 87, 104, 150, 152). To persist, viruses must also become biochemically adapted, so that they can replicate without severely compromising the host's survival.

Some viruses, including SARS-CoV, elicit an immune response in the nonreservoir host that may contribute to pathology (75, 148, 157) while apparently, at least for SARS viruses, not causing immunopathology in the reservoir. Elucidating the immune responses in reservoir hosts that determine the balance between virus persistence and immunopathology could contribute to our understanding of viral pathogenesis in humans and reveal potential targets for therapeutic intervention.

Some paramyxoviruses, including Nipah and Hendra viruses, encode V proteins that bind to signal transducer and activator of transcription 1 (STAT1) and STAT2 proteins of host cells to block both alpha/beta and gamma interferon responses (122). How the viral proteins might affect potential interferon responses to virus infections in bats is unknown. Possibly the V proteins play a role in viral persistence and evasion of the immune response. Addressing these important issues regarding the pathophysiology of viral infections in bats will require the development of infection models for reservoir species of each zoonotic virus.

With regard to bats, there is evidence that "healthy bats" can

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be asymptomatically infected with rabies virus. In nonlethal rabies infections produced in Mexican free-tailed bats, individuals surviving infection do not have virus in the brain or saliva. In one experimental study of free-tailed bats inoculated with salivary gland tissues from naturally infected bats, the incubation periods were 24 to 125 days, but one asymptomatic bat sacrificed at the end of the study had rabies virus in the brain, salivary glands, and other organs (11). Incubation periods for rabies virus are certainly highly variable in bats, and persistence of virus in hibernating bats has been suggested as serving a viral reservoir function (143). A carrier state for rabies virus has also been suggested by experimental and observational studies among dogs in Ethiopia (48).

Discovery of Emerging Viruses in Wildlife

It is, perhaps, instructive that viruses of the families *Paramyxoviridae*, *Filoviridae*, *Bornaviridae*, and *Rhabdoviridae* are phylogenetically related and have been grouped in a single order, the *Mononegavirales* (120). There are at least 250 recognized viruses in this order, including some that infect humans, other primates, livestock, birds, dogs, seals, fish, crabs, mosquitoes, ticks, amoebae, plants, or bats. If there is an unrecognized tendency for bats and viruses to be associated, viruses of this order would be prime targets for beginning the search.

Essentially every living life form investigated has been shown to host viruses, and bats are no exception. However, it is reasonable to query the roles of viruses of bats. What role, for example, does a fruit-eating bat play in the life cycle of a human or livestock pathogen? If human and livestock infections from bats simply are host-switching phenomena, why have these viruses not been recognized previously, and why have they emerged now? Are these events the results of ecologic alterations, such as impingement of human activities on heretofore virgin areas, consequences of global climate change, or the product of improved surveillance activities coincident with the technical advances in diagnostic capabilities required to identify heretofore undescribed zoonotic viruses?

Are viruses of bats symbionts, parasites, or commensals? Is pathogenicity for humans and livestock simply a freak occurrence? Perhaps these emerging bat viruses are naturally transmitted by arthropods or by other potential vectors that have not been examined. Surely a fatal infection in a host is not in the long-term best interest of the virus. Might fruit-eating bats transmit viruses to or from plants? How? Are insectivorous bats intermediate hosts between insects and vertebrates (or plants)? Are fruiting events part of periodic amplification cycles of viruses from frugivorous bats to wildlife and humans, as suggested by Dobson (42)? Childs summarized the processes by which zoonotic viruses are transmitted (29). He noted the rarity of surveillance for wildlife diseases or infections and suggested that such studies usually are outbreak-driven, i.e., after an epidemic of a newly recognized virus has emerged.

Outbreaks of Hendra virus, Nipah virus, Menangle virus, SARS-CoV-like viruses of bats, and European bat lyssavirus 1 (108) have not been recognized more than once or a few times. Are transmissions between bats and other vertebrates infrequent, incidental spillover events? Do many of the 66 viruses listed in Table 2 represent fortuitous, irrelevant events, or have we detected only the tip of the iceberg? Do bats differ from other mammals in their ability to clear viral infections? Does the persistence of asymptomatic viral infections in bats indicate that bats are an important reservoir for the wide variety of viruses in nature? Is the prevalence of RNA viruses in persistent infections in bats indicative of a defect in host resistance or viral clearance mechanisms, such as interferon or interferon-responsive genes that lead to clearance of RNA viruses from other vertebrates? There is some urgency to explore these important questions.

There is no reason to believe that bats are different from other mammals with regard to species specificity of host susceptibility to virus diseases, nonuniform persistence of viral infections, or mechanisms of virus shedding, so that such investigations likely do not require development of new assay systems or diagnostic concepts. Additional research is needed to determine the roles played by bats of various species in the natural histories of the viruses for which bats can serve as hosts.

One or more of the 66 viruses listed in Table 2 have been isolated from bats of 74 species. As well, viruses have been isolated from bats not identified further than to genus level and from four unidentified bats. Some viruses have been isolated from bats of as few as 1 species and one from as many as 14. Clearly, bat handlers, people entering bat habitat areas, and people who usually think in noninfectious disease terms regarding various studies of the bats themselves should take necessary precautions to avoid exposing themselves to recognized and unrecognized viruses and to other human pathogens which the bats may harbor.

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ADDENDUM IN PROOF

In addition to finding a recent publication reporting the indentification of six novel coronaviruses from six different bat species in Hong Kong alone (P. C. Woo, S. K. Lau, K. S. Li, R. W. Poon, B. H. Wong, H. W. Tsoi, B. C. Yip, Y. Huang, K. H. Chan, and K. Y. Yuen, Virology, Epub ahead of print, doi:10.1016/j.virol.2006.02.041, 2006), we have learned of other viruses isolated from or detected in bats from Africa and of many as-yet-unpublished viruses recently detected in bats in

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Australia. Thus, our article may contain only an indication of the great potential for future discoveries of viruses in bats worldwide.

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SOURCE REDUCTION IN FLORIDA'S SALT MARSHES: MANAGEMENT TO REDUCE PESTICIDE USE AND ENHANCE THE RESOURCE

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ABSTRACT. Source reduction as part of an integrated pest management program is a cornerstone of the American Mosquito Control Association's Pesticide Environmental Stewardship Program Strategy Document to reduce pesticide risk. Since the early 1980s, Florida has made important strides in implementing environmentally sound source reduction strategies in salt marshes while managing them for both mosquito control and natural resource enhancement. The political mechanism for this progress has been interagency cooperation through the Florida Coordinating Council on Mosquito Control and its Subcommittee on Managed Marshes. Challenges in accomplishing source reduction continue because both public and private lands are involved. Public lands include those owned by federal (e.g., U.S. Fish and Wildlife Service, National Park Service), state (Florida Department of Environmental Protection), and local governments, and they have a diversity of management objectives. This diversity adds to the challenge facing mosquito control agencies in providing mosquito control services while protecting and enhancing the environment.

KEY WORDS Salt marsh, source reduction, mosquito larval control, IPM

INTRODUCTION

Source reduction, as a method of mosquito control without the use of pesticides, can be as simple as removing tires, containers, and plants that provide breeding space for *Aedes* and *Wyeomyia* mosquitoes, to as complex as multipurpose salt-marsh management. In a salt-marsh management program, source reduction needs to be part of an integrated approach, typically also including larviciding and adulticiding. Source reduction is usually the most effective and economical of these 3 techniques for salt-marsh mosquito control.

In 1994, the Environmental Protection Agency (EPA), in association with the U.S. Department of Agriculture and the U.S. Food and Drug Administration, initiated the Pesticide Environmental Stewardship Program (PESP). PESP's primary goal is to reduce pesticide risk while promoting environmental stewardship. Three years later, the American Mosquito Control Association (AMCA) became a PESP partner. Environmentally sensitive salt-marsh management techniques, such as source reduction, by using an integrated pest management (IPM) approach meet PESP goals and are a cornerstone of the AMCA's PESP Strategy Document. The AMCA has further developed this relationship by allowing other mosquito control organizations that have demonstrated their commitment to reducing pesticide risk to apply to become "PESP partner(s) under the AMCA's auspices" (Carlson 1997). To date, 7 organizations have become such partners and deserve credit for making a significant commitment to environmental stewardship: 1) Mosquito & Vector Control Association of California, 2) Florida Mosquito Control Association, 3) Louisiana Mosquito Control Association, 4) New Jersey Mosquito Control Association, 5) North Carolina Mosquito & Vector Control Association, 6) Northeast Mosquito & Vector Control Association, and 7) Northwest Mosquito & Vector Control Association.

FLORIDA'S SALT-MARSH SOURCE REDUCTION HISTORY

Ditching as a salt-marsh mosquito control technique was common as early as the 1920s. These projects typically used evenly spaced ditches to dewater the marsh and did not consider marsh topography. Silting frequently closed the ditches along the estuary edge, thereby hindering their effectiveness. Even when properly maintained, the areas between ditches still produced mosquitoes. Pumping water out of mosquito-producing diked marshes was briefly attempted, but it was not effective because it was impossible to dewater the area before mosquitoes emerged. Marsh filling, although successful in eliminating mosquito production by creating uplands from wetlands, proved too slow and costly. The environmental problems associated with dredge and fill projects included loss of wetland habitat and increased estuarine turbidity with an associated loss in seagrasses.

Large-scale impounding of high marsh began in the mid-1950s by constructing earthen dikes around known mosquito-producing high marsh, allowing for water level manipulation. Flooding impoundments during the summer eliminates production of salt-marsh mosquitoes because

they will not lay their eggs on standing water. Impounding is the most widely used source reduction technique in Florida with >16,000 ha of salt marshes and mangrove swamps included in 192 impoundments, most of them along the Indian River Lagoon (IRL). Approximately 1/2 the impounded acreage occurs on the Merritt Island National Wildlife Refuge (MINWR) in Titusville. Much of the impounded acreage on the MINWR is managed for waterfowl (Carlson et al. 1991). Currently, a variety of impoundment management techniques are used at the east coast's impoundments. It is generally agreed that a mix of management techniques is most beneficial for the local ecology (Rey et al. 1999).

ENVIRONMENTAL EFFECTS OF IMPOUNDING

Research and management experience has shown that impounding can have both negative and positive effects on natural resources. Impoundment dikes prohibit access to aquatic organisms that must use the high marsh during a portion of their life cycle, which include several commercially and recreationally important fish species (snook, mullet, ladyfish, and tarpon) (Brockmeyer et al. 1997).

Although impounding is highly effective in controlling salt-marsh mosquitoes and can virtually eliminate the need for chemical control in impounded marshes (Carlson and O'Bryan 1988), excessive or prolonged flooding of these impounded habitats can stress or kill existing high marsh vegetation and significantly alter plant distribution and species composition. However, resident fish in impoundments can be more abundant than in native marshes. They, in turn, can provide an abundant food source for wading birds and other predators (Schooley 1980). MINWR has demonstrated enhanced marsh use by waterfowl and wading birds as a result of impounding (Provost 1959, Trost 1964). Balancing the effects of impounding is a challenge, and in the late 1970s and early 1980s, conflicts arose between mosquito control organizations and agencies responsible for salt-marsh habitats as to how these areas should be managed. Some resource agencies advocated removing impoundment dikes to return them to a more natural condition. Mosquito control agencies countered that by doing so, they would have to increase the use of larviciding to carry out their mandate.

GOVERNMENT REGULATIONS AND AGENCIES

Subcommittee on Managed Marshes

To try and amicably resolve this and other environmental disagreements, in 1983, then Governor (and later Senator) Bob Graham created two committees: the Florida Coordinating Council on Mosquito Control and its Subcommittee on Managed Marshes (SOMM). In 1986, both of these committees became formally established in the Florida Statutes within mosquito control legislation (Chapter 388).

The Subcommittee on Managed Marshes plays an advisory role to ensure that salt-marsh source reduction projects, whether being implemented by private or governmental entities, take into account both mosquito control and natural resource requirement. SOMM currently includes 13 members from agencies responsible for natural resources, research laboratories, and local governmental agencies. Members meet quarterly at different locations in Florida, with the meetings typically including a field trip to representative habitats of the area visited, followed by a business meeting. Based on research conducted since the early 1980s, the techniques that SOMM agrees best meet the dual roles of providing mosquito control with a minimum of pesticide use while allowing the marsh to function as naturally as possible are rotational impoundment management (RIM) and open marsh water management (OMWM) (Carlson et al. 1999). Most of the salt-marsh projects reviewed by SOMM during the 1990s and early 2000s have proposed either RIM or OMWM.

Rotational impoundment management: RIM is the most commonly used management technique in impoundments and involves the installation of culverts with flap-gated risers through impoundment dikes to seasonally reconnect the impounded marsh and estuary. Culverts are closed in the late spring; the marsh is minimally flooded by pumping during the summer to prevent salt-marsh mosquito oviposition. In the early fall, culverts are opened to the estuary, enabling the annual fall high tides to enter the marsh. Rotational impoundment management is generally agreed to be the best compromise impoundment management technique because it allows the marsh to perform most of its natural functions while reducing sources for mosquito breeding and enabling mosquito control with a minimum of pesticide use (Carlson et al. 1991).

Open marsh water management: During the 1990s, OMWM was increasingly implemented in Florida, especially along the northern IRL in Volusia and Brevard counties. Open marsh water management projects with rotary ditching have been implemented in impoundments where summer flooding was no longer deemed necessary and in disturbed marshes, typically where mosquito control ditching was done years ago and the ditches are no longer serving their optimal function. OMWM allows for mosquito producing areas on the marsh to be connected to deeper water habitats, thus facilitating drainage and

interrupting mosquito oviposition or conversely, allowing larvivorous fish access to mosquito larvae (Carlson et al. 1991).

Surface Water Improvement and Management (SWIM) Act

In 1987, the Florida legislature passed the SWIM Act with the goal of improving the management of Florida's surface waters. Unfortunately, state funds are no longer provided for this program; the SWIM program is currently being supported and administered by regional water management districts. Along the IRL, both the St. Johns River Water Management District and the South Florida Water Management District have taken an aggressive role in providing funding to purchase culverts with water control structures to allow the reconnection of impoundments to the lagoon. This support is usually entered into as a partnership with the water management district purchasing the structures and the local mosquito control agency installing them and being responsible for their future management and maintenance. Largely through these cooperative efforts, close to 75% of the entire IRL impoundment acreage has been reconnected (Brockmeyer, personal communication).

Salt-marsh management and research workshops

Beginning in 1988 at 4-year intervals, SOMM has sponsored 5 workshops designed to bring interested individuals up to date on topics of importance to salt-marsh managers. A collection of abstracts from each meeting has been published. The most recent meeting was held in February 2005 in Cocoa Beach, FL, and was held jointly with the 4th Biennial Mosquito Control Conference. The conference is designed to present findings on research and management specifically in Mosquito Lagoon, a lagoonal estuary and part of the IRL, in northern Brevard and southern Volusia counties, on property near the Kennedy Space Center and adjacent to the MINWR. The topics at this meeting included environmental and land use planning, a multidisciplinary research project called the "Wetlands Initiative," mosquito control issues, habitat restoration, and topics dealing with salt-marsh wildlife and aquatic organisms. An abstract collection was published as a Technical Bulletin of the Florida Mosquito Control Association.

MANAGEMENT OF PUBLICLY OWNED LANDS

Typically, mosquito control agencies do not own the lands that they manage. These properties include both public and private land. Public lands include federal properties operated by 1) U.S. Fish and Wildlife Service (e.g., MINWR [Brevard Co.], Ding Darling NWR [Lee Co.], Pelican Island National Wildlife Refuge [Indian River Co.]) and 2) National Park Service (e.g., Canaveral National Seashore [Brevard and Volusia counties], Everglades National Park [Dade and Monroe counties]). State lands include state parks (e.g., Sebastian Inlet [Indian River Co.], Jack Island [St. Lucie Co.]) and aquatic preserves (e.g., Charlotte Harbor, Rookery Bay [Collier Co.]). Local environmentally sensitive parks, nature trails, and preserves also may be managed for mosquito control and natural resource protection.

Significant efforts are underway to publicly purchase privately owned salt marshes as funding sources become available. Managing these areas for mosquito control is a challenge because the landowners can have different preferences for the techniques used on their properties. For example, in Florida, to conduct mosquito control on state lands, an "arthropod control plan" must be submitted and approved by the Florida Department of Environmental Protection (FDEP). The FDEP is generally supportive of source reduction by using RIM or OMWM and of larviciding by using *Bacillus thuringiensis* ssp. *israelensis* or methoprene. Private owners may or may not be supportive of these methods.

About 15 years ago, Carlson et al. (1991) described the importance of having salt marshes in public ownership to expedite the implementation of optimal management practices. This benefit remains true today. Although during the 1990s and early 2000s many Florida counties purchased numerous, sizable tracts of salt-marsh wetlands in collaboration with the state and regional water management districts, vast acreages still remain in private sector ownership. When such properties are placed under public ownership, they can be more appropriately managed than is now allowed by the current private owners and thus significantly benefit salt-marsh management efforts in Florida.

SUMMARY

The past 2.5 decades have seen tremendous progress in the fine-tuning and more widespread implementation of environmentally friendly source reduction techniques in Florida salt marshes. These efforts are consistent with the EPA's PESP and should be encouraged at all levels of government. Reducing pesticide risk and enhancing the environment while still protecting the health and comfort of our citizens is a goal toward which we should strive.

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Efficacy of Aerial Spraying of Mosquito Adulticide in Reducing Incidence of West Nile Virus, California, 2005

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Epidemic transmission of West Nile virus (WNV) in Sacramento County, California, in 2005 prompted aerial application of pyrethrin, a mosquito adulticide, over a large urban area. Statistical analyses of geographic information system datasets indicated that adulticiding reduced the number of human WNV cases within 2 treated areas compared with the untreated area of the county. When we adjusted for maximum incubation period of the virus from infection to onset of symptoms, no new cases were reported in either of the treated areas after adulticiding; 18 new cases were reported in the untreated area of Sacramento County during this time. Results indicated that the odds of infection after spraying were ≈6× higher in the untreated area than in treated areas, and that the treatments successfully disrupted the WNV transmission cycle. Our results provide direct evidence that aerial mosquito adulticiding is effective in reducing human illness and potential death from WNV infection.

West Nile virus (WNV; genus *Flavivirus*, family *Flaviviridae*) is transmitted to humans through the bite of an infected female mosquito and can cause clinical manifestations such as acute febrile illness, encephalitis, flaccid paralysis, and death (*1*). In California, WNV was first identified in 2003, during which time the virus was detected in 6 southern counties and 3 infected persons were identified (*2*). The following year, WNV spread northward from southern California to all 58 counties in the state, resulting in 779 human WNV cases and 28 deaths (*3,4*). In 2005, 880 human WNV cases and 19 related deaths were identified in California; 3,000 cases were reported nationwide (*5,6*). In contrast to 2004, when most of the WNV activity was concentrated in southern California, activity in 2005 occurred primarily in the northern part of the Central Valley of California, where Sacramento County, the epicenter of WNV activity in the United States that year, had more human cases (163) than any other county in the nation (*7*).

In northern California, the principal urban and rural vectors of WNV are *Culex pipiens* and *Cx. tarsalis*, respectively (*8–10*). To reduce WNV transmission and human exposure to mosquitoes in 2005, the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) implemented a battery of control practices from their Integrated Pest Management plan (*11*), an ecosystem-based strategy focused on long-term control of mosquito populations (D. Brown, SYMVCD, pers. comm.). Despite the district's intensified efforts (which began in March 2005) to control larval mosquitoes and to spot-treat for adult mosquitoes by using truck-mounted equipment, by August 2005 the county had reached the epidemic response level designated by the California Mosquito-Borne Virus Surveillance and Response Plan (*12,13*). Per the response plan, SYMVCD determined the appropriate response and control measures through the analysis of 8 surveillance factors, which provided a semiquantitative measure of transmission risk (D. Brown, pers. comm.). Rapidly escalating risk for WNV transmission to humans in Sacramento County was indicated by high mosquito abundance and infection prevalence; high numbers of sentinel chicken seroconversions;

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and record numbers of dead bird reports, equine cases, and human cases, including ≈24 confirmed human infections by early August (*8,10,14*). Following state guidelines, and in consultation with local public health officials, SYMVCD initiated aerial adulticiding in Sacramento County in August 2005 to rapidly reduce the abundance of infected mosquitoes and decrease the risk for WNV transmission to humans (D. Brown, pers. comm.). Despite a 60-year history of the aerial application of mosquito control products in California (*15*), this was the first instance within the state of aerial adulticiding over a large urban area.

Although published studies on aerial application of adulticides have documented reductions in mosquito abundance and infection prevalence along with concurrent or subsequent decreases in human cases (*16–19*), no published study to date has directly assessed the efficacy of such control efforts in reducing incidence of human disease by comparing distribution of clinical cases within treated and untreated areas. The objective of our study was to evaluate the efficacy of adulticide applications for reducing human cases of WNV; we compared the proportion and incidence of cases in the treated and untreated areas of Sacramento County in 2005 before and after aerial treatments. The proportion and incidence of these cases were also compared with those of the rest of California.

Methods

Data Collection

Human WNV case data were reported to the California Department of Public Health from the Sacramento County Department of Health and Human Services and other local health departments throughout the state by using a standardized case history form. A total of 177 human infections were reported within Sacramento County in 2005, with onsets of illness ranging from June through October. Of 177 infections, 163 were clinical cases and 14 were asymptomatic infections; the former was confirmed by immunoglobulin (Ig) G and IgM antibody assays of serum or cerebrospinal fluid samples. Of 163 case records, 7 had no date-of-onset information and 4 others had no residential address. Consequently, the Sacramento County human dataset used in this study comprised 152 records that contained spatial and temporal attributes.

Residential addresses were imported into ArcMap 9.1 geographic information systems software (Environmental Systems Research Institute, Inc., Redlands, CA, USA) and geocoded by using the software's 2005 StreetMap USA Plus AltNames street dataset. All remaining unmatched addresses were geocoded by using Tele Atlas 2006 (Tele Atlas, Lebanon, NH, USA), NAVTEQ 2006 (NAVTEQ, Chicago, IL, USA.), GDT 2005 (Geographic Data Technology, Inc., Lebanon, NH, USA), and TIGER 2006 (US Census Bureau, Washington, DC, USA) datasets. Population size estimates for the study areas defined below were calculated in ArcMap by selecting census blocks that had their center (centroid) in each defined region (Table 1) (*20*). All data were mapped by using the NAD83 USA Contiguous Albers Equal Area Conic coordinate system.

Adulticide Application

Aerial adulticide applications were intended to create aerosolized clouds of insecticide that would contact, and consequently kill, airborne adult *Culex* spp. mosquitoes. SYMVCD targeted areas for treatment on the basis of levels of mosquito infection prevalence that had been previously associated with epidemic transmission within an urban setting (minimum infection rate per 1,000 female *Culex* spp. tested >5.0) (*12*). The district contracted with ADAPCO Vector Control Services (ADAPCO, Inc., Sanford, FL, USA) to apply adulticide by using 2 Piper Aztec aircraft (Piper Aircraft, Inc., Vero Beach, FL, USA) over an area of 222 km^2 in northern Sacramento County on the nights of August 8–10, 2005 (northern treated area) and an area to the south of 255 km^2

Table 1. Number of human cases of infection with West Nile virus by location and temporal classification, California, 2005*

Area†	Total	Pretreatment‡	Posttreatment§	Postincubation¶	Population#
Treated, northern	34	28	6	0	221,828
Treated, southern	21	20	1	0	338,579
Buffer, northern	13	9	4	3	94,399
Buffer, southern	8	5	3	1	50,127
Untreated	76	41	35	18	518,566
Sacramento County	152	103	49	22	1,223,499
California	670	357	313	197	32,648,149

*Only cases with known date of onset of illness and location information (i.e., Sacramento County at the address level and California at the county level) are included in the analysis.
†California excluding Sacramento County.
‡Refers to cases with onset of illness up to and including the last date that aerial adulticiding was conducted (ending 22 Aug for the southern treated area and southern buffer zone and 10 Aug for all other areas).
§Refers to cases with onset of illness after the last date that aerial adulticiding was conducted (beginning 23 Aug for the southern treated area and southern buffer zone and 11 Aug for all other areas).
¶Refers to cases with onset of illness >14 days after the first date that aerial adulticiding was conducted (beginning 4 Sep for the southern treated area and southern buffer zone and 23 Aug for all other areas).
#Population data source: UA Census 2000 TIGER/Line data made available in shapefile format through Environmental Systems Research Institute, Inc. (Redlands, CA, USA) (*20*).

on the nights of August 20–22, 2005 (southern treated area) (D. Brown, unpub. data) (Figure 1). Coverage was similar each night; repeated applications were intended to increase efficacy (D. Brown, pers. comm.).

The applied compound was Evergreen EC 60–6 insecticide (MGK, Minneapolis, MN, USA), a product composed of 6% pyrethrin/60% piperonyl butoxide (*8*). It was applied at the maximum rate according to the label, 0.0025 pounds of pyrethrins per acre (ultra-low volume dispersal), by 2 Micronair AU4000 atomizer nozzles (Micron Sprayers, Ltd, Bromyard, Herefordshire, UK) on each aircraft, with a swath width of 1,300 feet and expected droplet spectrum volume mean diameters of 32.1 and 36.3 microns for the 2 planes (D. Brown and G. Goodman, unpub. data). Conditions during each night of spraying included wind speeds of 4–10 knots/h and temperatures/dew points of 27°C/14°C (northern treatment) and 33°C/12°C (southern treatment) (D. Brown, unpub. data). Planes began flying at ≈8:00 PM each night and flew for 3–6 h at 130 knots/h (D. Brown, unpub. data). The aircraft flew at altitudes of 61.0 m in the northern treated area and 91.4 m (because of obstacles such as tall towers and buildings) in the southern treated area (R. Laffey, SYMVCD, unpub. data, D. Markowski, pers. comm.). The Wingman GX aerial guidance and recording system (ADAPCO, Inc.), coupled with the Aircraft Integrated Meteorological Management System (AIMMS-20; Aventech Research, Inc., Barrie, Ontario, Canada), modeled the effective drift of released compounds on the basis of real-time meteorologic conditions (D. Brown, pers. comm.). Flight and treatment data were imported into ArcMap for mapping and analysis.

Figure 1. Map of northern and southern aerial adulticiding treatment areas in Sacramento County, California, 2005, showing the 2 urban areas treated by the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). Horizontal bars represent swaths of spray clouds created by individual passes of the aircraft, as defined by the spray drift modeling systems. Gaps within spray clouds were caused by factors such as towers and buildings that altered the flight of the aircraft (G. Goodman, SYMVCD, pers. comm.). These gaps were assumed to have negligible effect in this study; no human cases occurred within any gaps. Gray region surrounding much of the spray zones represents the urbanized area of Sacramento; urbanized area is defined by the US Census Bureau as a densely settled territory that contains ≥50,000 persons (*21*). For display purposes, we used the NAD83 HARN California II State Plane coordinate system (Lambert Conformal Conic projection). Inset shows location of treatment areas in California.

Case Classification and Analysis

Despite the spray drift modeling systems' high degree of accuracy, variable and incomplete spray application was expected at the edges of the modeled spray cloud (D. Markowski, pers. comm.). Factors contributing to this phenomenon include the intrinsic margin of error of the aircrafts' spray drift modeling systems, the extrinsic margin of error caused by factors not detectable or taken into account by the modeling system (i.e., wind gusts, minor changes in aircraft altitude or speed, and other operational variables), and nonoverlapping spray clouds during different nights of application (D. Markowski, pers. comm.). Through consultation with ADAPCO, Inc., this variable and incomplete application at the perimeter was taken into account by delineating a 0.8-km (0.5-mile) buffer within the outermost range of the modeled spray clouds for each treated area (D. Markowski, pers. comm.). Nonbuffered areas of the spray regions (henceforth referred to as treated areas) were considered the most accurate representation of the actual spray application for this analysis, and any WNV cases that occurred within buffer zones were considered separately from those within treated areas. All human cases from Sacramento County that did not occur within treated areas or buffer zones were assigned to the untreated subset of cases, which served as the comparison (control) group for this study.

Cases were further classified by date of onset of illness into pretreatment and posttreatment groups; temporal classification for the untreated area and the rest of California followed that of the northern treated area (Table 1). Because of the relatively lengthy and variable human WNV incubation period, persons who became infected just before the spray events could have become symptomatic up to 14 days later (*22,23*). To exclude from analysis any infections that may have been acquired just before the spray events, posttreatment cases that had an onset of illness >14 days after spraying (counting from the first night of application) were also included in a postincubation subset.

The null hypothesis, that the proportion of cases in treated and untreated areas was equal to that of the respective population size estimates, was tested for pretreatment and posttreatment groups with the exact binomial test for goodness of fit by using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html). Second, significance of proportions of human cases before and after spraying within treated and untreated areas was evaluated with the Fisher exact test of independence by using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The null hypothesis of this test was that there was no significant association between occurrence of adulticiding and temporal classification of cases (i.e., pretreatment or posttreatment). Third, relative risk (RR) and odds ratio (OR) of infection in the untreated area compared with those in treated areas were calculated by using cumulative incidence of WNV in each region before and after spraying (*24*). To evaluate whether buffer zones had any effect on results, all calculations were repeated by using cases from buffer zones and treated areas combined, as well as cases from buffer zones alone.

Assumptions

As is standard practice in most epidemiologic studies, residential addresses of patients were assumed to be locations of disease transmission; this is also consistent with other WNV studies (*25–31*). The assumption that WNV was transmitted to persons at their place of residence is supported by the fact that WNV mosquito vectors feed primarily from dusk to dawn, and also by findings that persons who spent >2 h outdoors during this time without wearing insect repellant had the highest WNV seroprevalence (*31*).

Because of the random sampling requirement for tests of statistical significance, we must assume that various human populations had an equal likelihood of becoming clinically ill before aerial treatment and that no preexisting factors contributed to a differential in disease experience. Although construction of a multilevel, spatial correlation model is beyond the scope of this study, several important properties of the populations sufficiently support our assumption of homogeneity. Despite the geographic size of the untreated area being ≈6× that of the treated areas combined (2,101 vs. 361 km^2, Figure 2), population size estimates of both areas were comparable (518,566 vs. 560,407, Table 1) (*20*). Furthermore, the preponderance of cases in the treated (100%, 55/55), buffer (95%, 20/21), and untreated (87%, 66/76) areas was located within the urbanized area of Sacramento, which constitutes 27% (686 of 2,578 km^2) of the total area of the county (Figure 1) (*20*). Additionally, most cases in the untreated area were located either between the northern and southern treated areas or immediately north of the northern treated area, and >94% (143/152) of all cases were located within 4.8 km (3 miles) of treated areas. This staggered configuration of treated and untreated areas, along with the general proximity of cases within 1 urban region, supported the assumption of homogeneity of populations at risk and created a natural experiment for comparative analyses between treated and untreated areas.

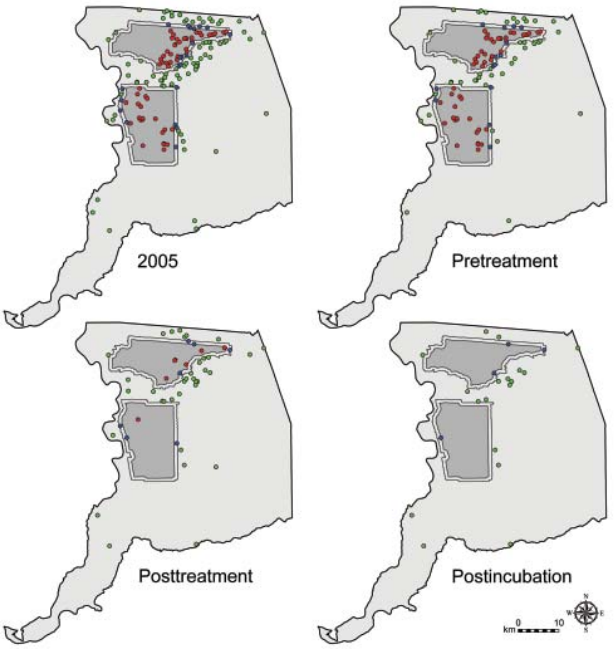

Figure 2. Locations of treated areas and human cases of West Nile virus by temporal classification, Sacramento County, California, 2005. Shown are treated areas (dark gray), surrounding 0.8-km buffers (thin regions around dark gray areas), untreated areas (light gray), and location of human cases within each of these regions (red, blue, and green circles, respectively). For display purposes, we used the NAD83 HARN California II State Plane coordinate system (Lambert Conformal Conic projection).

Results

The observed proportion of pretreatment cases in treated areas to those in the untreated area was not significantly different from the expected proportion on the basis of population size estimates (p = 0.7508, Table 2). Similarly, none of the proportions of pretreatment cases in any combination of treated areas and buffer zones were different from those of the untreated area. However, after adulticiding, all proportions of cases in treated areas were lower than that in the untreated area. Proportions of posttreatment cases in buffer zones were not different from those in the untreated area.

There was a significantly lower proportion of posttreatment cases within combined treated areas compared with that in the untreated area (p<0.0001, Table 2). Proportions of posttreatment to pretreatment cases within each of the individual treated areas were also significantly lower than that for the untreated area (northern treated area p = 0.0053; southern treated area p = 0.0003). After com-

Table 2. Statistical test results for West Nile virus cases, Sacramento County, California, 2005*

Area	Goodness of fit†		Independence‡
	Pretreatment	Posttreatment	Posttreatment vs. pretreatment
Treated, both	0.7508	**<0.0001**	**<0.0001**
Treated, northern	0.0650	**0.0391**	**0.0053**
Treated, southern	0.2983	**<0.0001**	**0.0003**
Treated plus buffer, both	0.6195	**<0.0001**	**0.0005**
Treated plus buffer, northern	0.1015	**0.0314**	**0.0069**
Treated plus buffer, southern	0.4568	**<0.0001**	**0.0029**
Buffer, both	0.5140	0.5744	0.3309
Buffer, northern	0.5592	0.5065	0.3745
Buffer, southern	0.5990	1.0000	0.7237

*Numbers of cases were combined for multiple areas; geographically corresponding buffer zones were added where noted. Numbers are 2-tailed p values. Statistically significant associations (p<0.05) are in **boldface**.
†Exact binomial goodness-of-fit test for observed proportion of cases in listed area(s) to cases in untreated area compared with the expected proportion based on population size estimates.
‡Fisher exact test of independence for 2 × 2 contingency tables containing numbers of pretreatment and posttreatment cases for listed area(s) and the untreated area.

bining cases from treated areas and buffer zones, proportions of posttreatment versus pretreatment cases were again significantly lower (both treated areas plus buffers p = 0.0005; northern treated area plus buffer p = 0.0069; southern treated area plus buffer p = 0.0029). However, none of the proportions of posttreatment versus pretreatment cases in buffer zones alone compared with those in the untreated area were significantly different (both buffer zones p = 0.3309; northern buffer zone p = 0.3745; southern buffer zone p = 0.7237).

The last human case that occurred in treated areas had an onset of illness 12 days after inception of spraying, within the 14-day maximum range of the human WNV incubation period. Thus, when the incubation period was taken into account, there were no new human WNV cases reported in either treated area after adulticiding (postincubation cases, Table 1, Figure 3). In contrast, 18 new cases were reported from the untreated area during this time; the last case occurred 59 days after inception of spraying. The frequency of these postincubation cases relative to the overall number of cases in the untreated area (24%) was consistent with that for the rest of the state (29%) but inconsistent with that for treated areas (0%).

Normalizing number of cases in each region by respective population size estimate showed the increase in incidence levels throughout the year (Figure 4). Statewide (excluding Sacramento County and cases without onset data), cumulative incidence in 2005 was 2.1/100,000 population, and the temporal pattern of incidence throughout the year was similar to that of the untreated area. On the basis of cumulative incidence within each region before aerial treatment, RR for the untreated area compared with that for treated areas was 0.9231 (95% confidence interval [CI] 0.6085–1.400), which did not differ from unity. After treatment, RR was 5.403 (95% CI 2.400–12.16), with an OR of 5.853 (5.403/0.9231, 95% CI 2.351–14.58) in favor of infection in the untreated area than in treated areas; RR and OR differed from unity. Similarly, RRs for the untreated area compared with those for treated areas and buffer zones combined were 0.8990 (95% CI 0.6059–1.334) and 3.398 (95% CI 1.829–6.316) before and after adulticiding, respectively, with an OR of 3.780 (3.398/0.8990, 95% CI 1.813–7.882). Conversely, RRs for the untreated area versus the buffer zones alone were 0.8162 (95% CI 0.4450–1.497) and 1.393 (95% CI 0.6190–3.137) before and after adulticiding, respectively, with an OR of 1.707 (1.393/0.8162, 95% CI 0.6198–4.703); the RRs and OR did not differ from unity.

Discussion

Evaluation of efficacy is essential for assessing appropriateness of insecticide applications. However, such studies assessing the ability of adulticides to directly affect human incidence of WNV have been nonexistent. Our findings, coupled with corroborating evidence of a reduction in the abundance of *Cx. pipiens* (*8*), indicate that aerial application of pyrethrin in 2005 successfully disrupted the WNV transmission cycle, and that this treatment was responsible for an abrupt decrease in the number of human cases within treated areas compared with that in the untreated area. These results provide direct evidence that aerial spraying to control adult mosquitoes effectively reduced human illness and potential deaths from WNV infection.

With respect to population size estimates, proportions of pretreatment cases in all treated areas and buffer zones were not different from that in the untreated area, which validates comparability of the baseline populations. Similarly, none of the pretreatment RRs deviated from unity, which supports the assumption that treated and untreated areas had an equal likelihood, on the basis of population size, of containing a clinical case before the adulticiding, and that no preexisting factors contributed to differing disease incidence rates during that time. These conditions are important for verifying that the untreated area was a valid

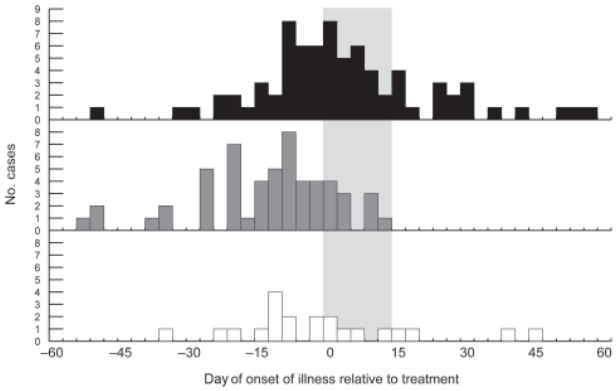

Figure 3. Human cases of West Nile virus (WNV), Sacramento County, California, 2005, by region and date of onset of illness. Black bars show cases within untreated area; gray bars show cases within northern and southern treated areas combined; and white bars show cases within northern and southern buffer zones combined. Values along the x-axis (days) are grouped into sets of 3 and labeled with the date farthest from 0. Each of the 3 days of adulticiding within the treated areas and buffer zones was considered to be 0; for the untreated area, the dates of the northern adulticiding (August 8–10) were considered to be 0. The wide gray vertical band represents time from the first day of treatment to the maximum range of the human WNV incubation period 14 days later.

comparison group for use in statistical analyses.

Comparisons of buffer zones with the untreated area indicated no differences between posttreatment RR or the proportions of posttreatment cases within the 2 areas, which supports the assumption of reduced spray efficacy at the perimeter of the modeled spray cloud. This finding may have implications for future aerial applications and efficacy studies. Additionally, posttreatment infiltration of *Cx. tarsalis* mosquitoes from bordering untreated areas has been a previously documented phenomenon in California and Texas (*19,32–34*). On the basis of mean dispersal distances of *Cx. tarsalis* (0.88 km) and *Cx. pipiens quinquefasciatus* (1.10 km) in California (*35*), use of the 0.8-km buffer in this study also reduced the probability of including in the treatment groups any human infections contracted through posttreatment mosquito infiltration. However, results of all statistical tests remained unchanged after combining the number of cases from buffer zones and treated areas, and these posttreatment reductions of cases still differed from that in the untreated area (Table 2).

Because posttreatment proportions of cases were lower than in the untreated area, we rejected the null hypothesis of goodness-of-fit comparisons. Our results also indicate that there were associations between adulticiding and temporal classification of cases. Therefore, we also rejected the null hypothesis of tests of independence. Furthermore, odds of infection after spraying were ≈6× higher in the untreated area than in treated areas. Without applications of aerial adulticide, more Sacramento residents would have been infected with WNV. This finding supports federal and California WNV response recommendations, which state that "mosquito adulticiding may be the only practical control technique available in situations where surveillance data indicate that it is necessary to reduce the density of adult mosquito populations quickly to lower the risk of WNV transmission to humans" (*36*).

Although there was a negative correlation between aerial treatments and incidence of human cases, causation is predicated upon spraying having a direct effect on mosquito populations. Recent work showed that adulticiding immediately reduced abundance and infection rates of *Culex* spp. mosquitoes compared with rates in an untreated area (*8*). Using factorial 2-way analysis of variance, these researchers compared mean abundances of *Cx. pipiens* and *Cx. tarsalis* from CO_2-baited traps (46 trap nights) in the northern treated area with mean abundances from traps (55 trap nights) in similar urban-suburban habitats within the untreated area of Sacramento County and adjacent Yolo County, 1 week before and 1 week after the August 8 spraying. Abundance of *Cx. pipiens* decreased by 75.0%, and there was a significant interaction between adulticiding and temporal classification (F 4.965, df 1,47, p = 0.031).

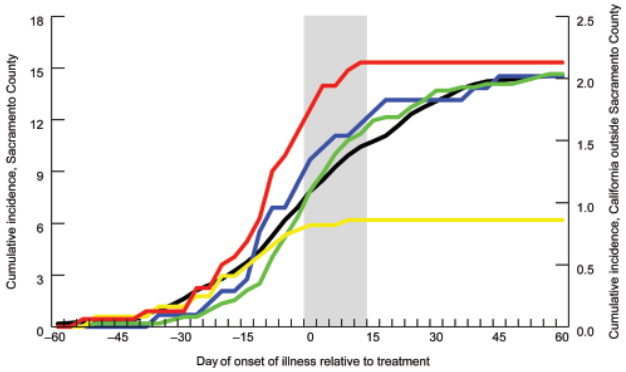

Figure 4. Cumulative incidence of human cases of West Nile virus (WNV) in Sacramento County and California, 2005. Only cases with known date of onset of illness and location information (i.e., Sacramento County at the address level and California at the county level) are included in the analysis. Cumulative incidence is the total no. WNV cases/100,000 population. Green line shows incidence within untreated area; red line shows incidence within northern treated area; yellow line shows incidence within southern treated area; blue line shows incidence within northern and southern buffer zones combined; black line shows incidence within California, excluding Sacramento County. Values along the x-axis (days) are grouped into sets of 3 and labeled with the date farthest from 0. Each of the 3 days of adulticiding within the treated areas and buffer zones was considered to be 0; for the untreated area and the rest of California, the dates of the northern adulticiding (August 8–10) were considered to be 0. The wide gray vertical band represents time from the first day of treatment to the maximum range of the human WNV incubation period 14 days later.

Abundance of *Cx. tarsalis* decreased by 48.7% but the interaction was not statistically significant (F 0.754, df 1,47, p = 0.390). As stated by these researchers, this disparity may have been caused by the presence of "an increasing population of *Cx. pipiens* and an already declining population of *Cx. tarsalis*" at the time of the spraying, and because *Cx. tarsalis* breeds principally in rural areas. Regardless, we reason that *Cx. pipiens* was the primary vector in the Sacramento County epidemic because this species is the principal urban vector in this region (*8–10*), was the most abundant species collected in Sacramento County in 2005 (D.-E.A. Elnaiem, unpub. data), and comprised the highest percentage of WNV-infected mosquito pools (68.3% versus 28.8% for *Cx. tarsalis*) in Sacramento County that same year (*10*).

Additionally, these researchers combined mosquitoes of both species (into pools of ≤50 females) taken from aforementioned traps and others in the northern treated area and untreated area 2 weeks before and 2 weeks after the August 8 adulticiding. Pools of mosquitoes were tested for WNV by using a reverse transcription–polymerase chain reaction, and infection rates were calculated by using a bias-corrected maximum likelihood estimation (www.cdc.gov/ncidod/dvbid/westnile/software.htm). After spraying, infection rates decreased from 8.2 (95% CI 3.1–18.0) to 4.3 (95% CI 0.3–20.3) per 1,000 females in the spray area and increased from 2.0 (95% CI 0.1–9.7) to 8.7 (95% CI 3.3–18.9) per 1,000 females in the untreated area. Furthermore, no additional positive pools were detected in the northern treatment area during the remainder of the year, whereas positive pools were detected in the untreated area until the end of September (D.-E.A. Elnaiem, unpub. data). These independent lines of evidence corroborate our conclusion that actions taken by SYMVCD were effective in disrupting the WNV transmission cycle and reducing human illness and potential deaths associated with WNV.

Historically, human WNV cases in the United States peak in August (*37,38*). This pattern was observed in Sacramento County and the rest of California in 2005, in which 61% (93/152) and 47% (314/670), respectively, of human cases had onset of illness in August. The next highest month was July, during which 27% (41/152) and 29% (195/670) of human cases had onset of illness in the county and the rest of the state, respectively. These findings are consistent with others from Sacramento County in 2005, which indicated that mosquito infection rates peaked in July and August (*10*). Considering early summer amplification within vector populations and length of the human incubation period, WNV remediation efforts would be more effective in limiting illness and death associated with human infection if conducted at the onset of enzootic amplification rather than after occurrence of human cases.

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TOLERANCE THRESHOLDS FOR *AEDES ALBOPICTUS* AND *AEDES CASPIUS* IN ITALIAN URBAN AREAS

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ABSTRACT. With nuisance mosquito species, the goal of integrated pest management is to keep mosquito density below a tolerance level that is often set by economic, ecological, and political factors. This study compares actual human annoyance, as measured by a phone survey, with several measures of mosquito abundance, in order to determine a threshold that is both relevant and practical. The efficiency of CO_2-baited traps, container index (CI), and oviposition traps for monitoring *Aedes albopictus*, and CO_2-baited traps for monitoring *Aedes caspius*, was evaluated. CO_2-baited traps were confirmed to be of low efficiency in *Ae. albopictus* collection, while correlation matrices showed a good relationship between CI and the number of eggs collected ($R = 0.91$), and between number of eggs and phone-survey nuisance level estimates ($R = 0.88$). Correlation between CI and phone-survey nuisance levels was slightly lower ($R = 0.78$). We found a close relationship between the nuisance level declared by residents and mosquito captures obtained with CO_2-baited traps (*Ae. caspius*) and ovitraps (*Ae. albopictus*). An equation is presented to estimate annoyance according to dwelling characteristics and to the presence of children in the family.

KEY WORDS Threshold, *Aedes albopictus*, *Aedes caspius*, mosquito, monitoring, CO_2-baited trap, oviposition trap, container index

INTRODUCTION

In Italy, mosquito control was entirely devoted to decreasing nuisance, as vector activity in this area was considered negligible up to summer 2007, when an epidemic of Chikungunya virus occurred (Angelini et al. 2007). The primary indigenous nuisance species is *Aedes caspius* (Pallas). This floodwater species colonizes rice fields, pastures, inland and coastland marshes, irrigation canals, lagoons, and other sites. *Ae. caspius* is a crepuscular, outdoor biting species, strongly affecting economic activities in tourist localities. Recently, *Ae. albopictus* (Skuse), an exotic species imported via the trade in used tires, is rapidly spreading mainly in urban areas (Romi et al. 1999, Urbanelli et al. 2000, Carrieri et al. 2003). *Ae. albopictus* is an urban-inhabiting, daytime biting species, showing high anthropophily, and when populations achieve high densities, they strongly affect outdoor activities. Currently, *Ae. albopictus* and *Ae. caspius* may be regarded as the key mosquito pest species in Italy and require the adoption of specific control measures and, when possible, well-designed control programs.

Worldwide surveillance methods for *Ae. aegypti* and *Ae. albopictus* include the house (or premises) index (HI)—the percentage of houses infested with larvae and/or pupae; the container index (CI)—the percentage of water-holding containers infested with active immatures; and the Breteau index (BI)—the number of positive containers per 100 houses (PAHO 1994). In Italy, autochthonous mosquito species are regularly monitored using CO_2-baited traps. In 2003, we developed a new CO_2-baited trap called CAA2003-T to improve data collection quality. *Ae. albopictus* monitoring is currently conducted by counting the eggs in ovitraps (Fay and Eliason 1966, Hawley 1988) or in terms of simple positive/negative ovitraps (Bellini et al. 1996). Ovitrap data have also been successfully used to monitor the impact of various types of control measures in Italy (Carrieri et al. 2006). Ovitrap data have been reported to be sensitive in detecting low populations of *Ae. albopictus* (Marques et al. 1993, Romero-Vivas and Falconar 2005, Richards et al. 2006).

Focks (2003) pointed out that the number of eggs collected by ovitraps is influenced by local environmental factors that prevent estimating population density in specific sites. In this work, we try to investigate the reliability of population density estimates made by ovitrap data when positioned in an urban area of 1 km^2. The 2 species considered in this work have major ecological differences that must be taken into

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account when planning monitoring: *Ae. albopictus* is a container-breeding weak flyer with scattered densities inside the urban area, while *Ae. caspius* is a floodwater species with a greater dispersal flight ability and is capable of suddenly invading an entire urban area.

The human annoyance threshold represents the highest biting density that most citizens in a community find tolerable. The actual perceived nuisance, understood as being the tolerable number of human bites during a prefixed time period, has been found to be extremely variable within populations in previous studies (Headlee 1932, John et al. 1987, Morris and Clanton 1988, Read et al. 1994). The level of nuisance is determined not only by the mosquito density, which usually increases from urban to rural areas, and by the species of mosquitoes but also by socioeconomic and psychological factors (Morris and Clanton 1989, 1992; John et al. 1987).

A working value for treatment threshold is often based on the experience of mosquito control agencies, taking into consideration economic, ecological, and political factors. In major mosquito control programs in Italy, the thresholds are used to start adulticiding and are currently empirically defined as the number of females caught in CO_2-baited traps during a single night, being fixed at 30–126 (*Aedes–Culex*) in the Metapontino (Carrieri et al. 2005) and 52–565 (*Aedes–Culex*) in Comacchio (Pantaleoni 1996). The Piedmont Regional Law L.R.75/95, with the related guidelines DGR 67-9777 (PRB 1995, 2003), indicates the threshold of tolerance (TT) as the level of mosquito density in a territory that it is possible to consider acceptable for full enjoyment of environmental resources (Bellini 2003). This is expressed by the formula

$$\mathrm{TT} = \log\left(NA + NB^{0.70} + NC^{0.61} + 1\right) > 1.50,$$

where NA is the number of *Aedes* and *Ochlerotatus* females, NB the number of *Anopheles* and *Culex modestus* Ficalbi females, and NC the number of *Culex pipiens* L. and *Culiseta* females captured with CO_2-baited traps in one night. This equation includes the cumulative effect of the main nuisance mosquito groups being targeted as follows:

$$Aedes,\ Ochlerotatus = 32 \text{ females/trap/night},$$

$$Cx.\ modestus,\ Anopheles = 150 \text{ females/trap/night},$$

$$Cx.\ pipiens,\ Culiseta = 300 \text{ females/trap/night}.$$

In this study we evaluated the efficiency of some *Ae. albopictus* monitoring methods and we propose a system useful for assisting the decision-making process in *Ae. albopictus* and *Ae. caspius* control campaigns.

MATERIALS AND METHODS

The study was carried out in 2 1-km^2 areas in the town of Rovigo (50,289 inhabitants) and in the village of Villadose (5,248 inhabitants) in the province of Rovigo, northeast Italy. Each 1-km^2 area was subdivided in 9 zones of about 11 ha. The population density was 461 inhabitants/km^2 in Rovigo, and 161 inhabitants/km^2 in Villadose. The investigation was repeated 5 times during the 2003 season: June, beginning of July, end of July, August, and September. In both the areas, phone interviews were made and mosquitoes were monitored by means of CAA2003-T traps (CO_2 traps), ovitraps, and field inspections (CI).

In any monitoring system, the choice of how many samples to take is important, as the cost of sampling has a major effect on the sampling plan. Cost of monitoring (C) could be defined as

$$C = C_f + C_1 \times N,$$

where C_f represents the fixed costs and C_1 the cost of a single sample. Therefore, knowing the budget limits, it is possible to calculate the number of samples (N):

$$N = \left(C - C_f\right)/C_1.$$

Four monitoring methods were evaluated: ovitraps, CI, CO_2-baited traps, and telephone survey. The number of samples for each was fixed to have similar costs for the 4 methods. Table 1 shows the sample sizes adopted and relative costs.

Table 1. Sample size and relative costs[1] of the 4 monitoring methods used.

	Operational costs[2]	Material costs[3]	Total costs	Theoretical sample size	Adopted sample size
CAA2003-T trap	1	1	2	3.0	3
Ovitrap	0.75	0.01	0.76	7.9	7–8
Container index	0.3	0	0.3	20.0	19–21
Phone survey	0.1	0.001	0.101	59.4	51–89

[1] 1 = the cost of 1 job-hour.
[2] Including displacement, station choice, specimen collection, and determination.
[3] Including trap, lab apparatus, and CO_2.

The phone survey was conducted on a population sample of 207 families, 118 in Rovigo and 89 in Villadose. The sample drawn represented about 4% of the families living in each 1-km^2 area under study (number of families in each area was about 3,500 in Rovigo and 1,500 in Villadose (ISTAT 2003). The samples were randomly drawn from each zone (equally within zones) and the families were always the same during the 5 interviews. Phone calls were conducted at 12:00 p.m.–2:00 p.m. and 5:00 p.m.–7:00 p.m., when the probability of finding adult results was higher.

The socioeconomic parameters surveyed with the questionnaire were: age of the interviewed people; type of dwelling (independent house or block of flats, floor of the building, and garden); and family composition (presence of children <5 years). The questions relating to mosquitoes included which protection tools used (insecticide, net, and prevention); how many bites considered as tolerable or intolerable; change of behavior in the use of green spaces caused by mosquitoes and the daily time of nuisance; and level of annoyance experienced the day before the interview, choosing among 4 possibilities:

- *Inesistente*—Nonexistent (no experience of mosquitoes)
- *Sopportabile*—Tolerable (presence of mosquitoes, but not so important as to affect normal outdoor activities)
- *Forte*—Strong (high trouble but not so heavy as to interrupt outdoor activities)
- *Insopportabile*—Intolerable (when the mosquitoes prevent any outdoor activity)

The phone interviews were carried out by a single interviewer to increase consistency. The annoyance level assessment was recorded as a categorical numerical value, with 0 for Nonexistent, 1 for Tolerable, 2 for Strong, and 3 for Intolerable.

Mosquito sampling was conducted as follows. In each of the 1-km^2 areas, 3 CAA2003 CO_2 traps (Centers for Disease Control and Prevention–style trap with dry-ice container painted black to increase attractiveness to *Ae. albopictus*) were positioned in private gardens about 300 m apart and activated from 5:00 p.m. on the day before the phone interview to 9:00 a.m. on the day of the phone interview. An ovitrap consisting of a black plastic container of 400-ml volume, with a masonite oviposition strip (Bellini et al. 1996), was placed in each of the 9 zones within the areas, except for one zone without houses and one missed datum, for a total of 16 traps. These were activated and checked weekly from June 15 to September 13. As *Ae. albopictus* females typically take 3–7 days from bloodfeeding to oviposition (Briegel and Timmermann 2001), in the analysis the data from phone interviews were compared to the average of collected eggs the week following the interview in the zone. We preferred to use the average of eggs in the 1-km^2 area instead of the single ovitrap collection, which is poorly correlated with the density of females (Focks 2003, Richards et al. 2006). The CI data were collected the day following the phone interview by inspecting 3 premises per zone (chosen by a separate randomization) in a fixed time of 20 min per premises.

Analysis of the efficiency of the various sampling methods was done using relative variation (RV), defined as (standard error of the mean)/mean (Service 1993). According to Southwood (1978), an RV <0.25 is usually adequate for most extensive sampling surveys, although in certain intensive programs an RV <0.10 may be required. A highly aggregated population will likely produce a higher RV.

Phone-survey data were analyzed by blocks using analysis of variance and Duncan's test for mean separation. Analysis of the relationship between public survey results and mosquito numbers was done using stepwise regression, consisting in the removal of 1 variable at a time, according to the smaller loss of explaining variability. In order to analyze the qualitative variables related to the socio-demographic factors, they were turned into categorical ("dummy") variables (Zar 1984, Fabbris 1997). Because of the heavy *Ae. caspius* population present at the end of July, we did not use this data set in the comparison of mosquito population monitoring methods.

RESULTS

Mosquito population estimates

The CO_2 traps collected 19,905 hematophagous insects, of which 18,937 (95.1%) were *Cx. pipiens*, 817 (4.1%) *Ae. caspius*, 21 (0.1%) *Ae. albopictus*, and 130 (0.65%) Ceratopogonidae (Table 2). The densities of *Ae. caspius* and *Cx. pipiens* were much higher in Villadose than in Rovigo. The seasonal dynamic showed a clear peak in *Ae. caspius* population density in the 2nd half of July to be related to irrigation practices in the surrounding rural area.

Oviposition traps collected a total of 23,228 *Ae. albopictus* eggs in 13 wk of monitoring. Assuming container availability was similar in the 2 areas, ovitrap data showed the population density was higher in Rovigo than in Villadose, as expected since the colonization process has progressed further in Rovigo. Variability was lower in Rovigo than in Villadose during the whole season (Table 3).

The container inspections were conducted at 190 premises, of which 159 resulted positive with a total of 450 containers with water, of which 179 (39.7%) containers had *Ae. albopictus* larvae. The

Table 2. Seasonal dynamics of mosquito and ceratopogonid collections obtained with CO_2-baited traps.

		Month	Valid *N*	Mean	SD
Rovigo	*Cx. pipiens*	Jun.	3	285.00	86.97
		Jul1[1]	3	390.00	212.54
		Jul2	3	579.33	68.15
		Aug.	3	121.67	48.44
		Sep.	3	34.33	24.19
	Ae. caspius	Jul1	3	0.67	1.15
		Jul2	3	77.33	93.72
		Aug.	3	2.67	3.06
		Sep.	3	1.33	1.15
	Ae. albopictus	Aug.	3	2.33	3.21
		Sep.	3	1.00	1.00
	Ceratopogonidae	Jul1	3	8.00	13.86
		Jul2	3	1.67	2.08
		Aug.	3	2.00	3.46
Villadose	*Cx. pipiens*	Jun.	3	532.33	217.79
		Jul1	3	1,793.67	938.53
		Jul2	3	1,887.67	768.45
		Aug.	3	515.67	157.13
		Sep.	3	172.67	69.76
	Ae. caspius	Jun.	3	4.00	4.58
		Jul1	3	3.00	2.00
		Jul2	3	163.00	35.04
		Aug.	3	18.00	25.12
		Sep.	3	2.33	0.58
	Ae. albopictus	Aug.	3	1.33	1.53
		Sep.	3	2.33	2.08
	Ceratopogonidae	Jul1	3	4.33	5.13
		Jul2	3	19.33	21.46
		Aug.	3	5.67	3.21
		Sep.	3	2.33	1.53

[1] Jul1, sampling period at the beginning of July; Jul2, sampling period at the end of July.

Table 3. Seasonal dynamic of container index and relative variation (RV).

	Month	*N*[1]	Mean	SD	RV
Rovigo	Jun.	20	0.13	0.21	0.37
	Jul1[2]	22	0.19	0.32	0.36
	Aug.	21	0.57	0.41	0.16
	Sep.	20	0.55	0.35	0.14
Villadose	Jun.	19	0.11	0.26	0.57
	Jul1	19	0.16	0.34	0.48
	Aug.	19	0.28	0.36	0.29
	Sep.	19	0.21	0.32	0.34

[1] Premises with no active containers not included.
[2] Jul1, sampling period at the beginning of July.

Table 4. Seasonal dynamic and relative variation (RV) of the mean number of eggs/ovitrap/week.

		Valid *N*	Mean	SD	RV
Rovigo	Jun.	8	42.00	30.64	0.26
	Jul1[1]	8	149.25	130.51	0.31
	Jul2	8	142.75	82.48	0.20
	Aug.	8	274.88	66.79	0.09
	Sep.	8	215.50	59.47	0.10
Villadose	Jun.	8	9.38	11.96	0.45
	Jul1	8	57.00	96.86	0.60
	Jul2	8	68.38	68.93	0.36
	Aug.	8	108.56	70.92	0.23
	Sep.	7	140.29	102.84	0.28

[1] Jul1, sampling period at the beginning of July; Jul2, sampling period at the end of July.

RV of CI (Table 4) showed values higher than 0.25 early in the season, indicating a highly aggregated population, while RV values decreased to 0.14–0.16 during August and September in Rovigo where *Ae. albopictus* had well established and increased to 0.29–0.34 in Villadose where the species was still colonizing the area.

Correlation matrices of the samples of June, July 1, August, and September showed a good correlation (Table 5) between CI and the number of eggs collected ($R^2 = 0.82$) and between number

Table 5. Pearson product–moment correlations between container index (CI), ovitrap, and phone survey in Rovigo and Villadose.[1]

	CI	Ovitrap	Phone survey
CI	1.0000	0.9056*	0.7804*
Ovitrap	0.9056*	1.0000	0.8822*

[1] Significant correlations ($P \leq 0.05$) are indicated with an asterisk.

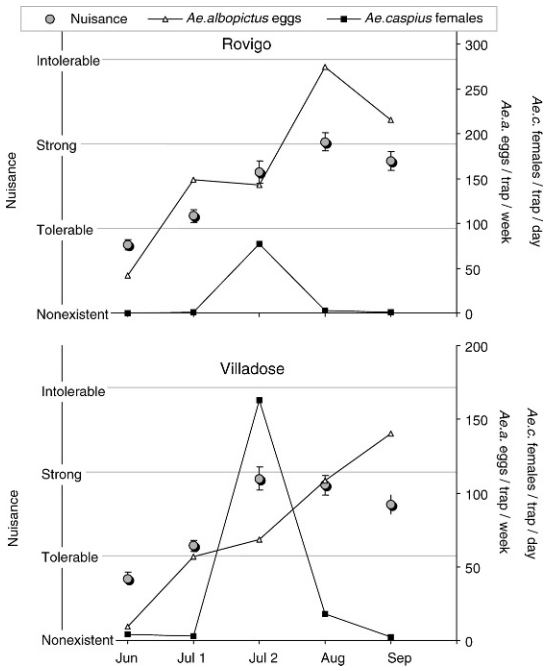

Fig. 1. Seasonal variation of the nuisance and captures of *Ae. caspius* females carried out with CO_2-baited traps and average number of *Ae. albopictus* eggs.

of eggs and phone survey ($R^2 = 0.78$), while lower correlation was observed between CI and phone-survey data ($R^2 = 0.61$). Correlations between CI and ovitrap data with the phone survey may have been affected by the presence, even at low density, of *Ae. caspius*.

Phone survey results

Altogether, 1,035 phone calls were made, producing 743 interviews (292 people were absent), including 415 people (some people answered more than one interview during the survey).

The mosquito annoyance seasonal dynamic showed a peak in Villadose at the end of July, related to the increase in *Ae. caspius* density, while in Rovigo it remained mostly related to the density of *Ae. albopictus* (Fig. 1).

The stepwise regression analysis of the data (mean of each area) showed that the surveyed nuisance level is strictly linked to the average density of *Ae. caspius* captured with the CO_2 traps (Table 6) and to the average density of *Ae. albopictus* eggs. The captures of *Cx. pipiens* and Ceratopogonidae did not seem to be consistent in citizens' annoyance assessment. The equation describing the relationship between nuisance level and mosquito monitoring was

$$M = 0.765 + 0.006Ae.c. + 0.004E_{Ae.a.} \quad (R^2 = 0.87, F_{2,7} = 23.13, P < 0.001), \tag{1}$$

where M is the surveyed nuisance, *Ae.c.* indicates the number of *Ae. caspius* females captured in 1 night/trap, and $E_{Ae.a.}$ is the average number of eggs collected weekly.

The analysis of the interview data highlighted that the nuisance expressed by the people was different according to various socioeconomic factors (Table 7). Women seemed to be more sensitive than men ($F = 8.46$, $P < 0.05$), even if differences between the number of bites considered tolerable ($F = 0.43$, $P = 0.55$) and intolerable ($F = 0.12$, $P = 0.75$) were not significant. Generally, the average number of bites considered tolerable by the interviewed people was 3.44 and 3.47 bites/day (respectively, among women and men); it was intolerable starting from 5.04 to 5.15 bites/day.

We did not observe significant differences in nuisance level ($F = 1.34$, $P = 0.31$) or in the number of tolerable ($F = 0.15$, $P = 0.93$) and intolerable bites ($F = 0.43$, $P = 0.73$) according to age, although reported nuisance level tended to decrease as age increased.

Among people living in independent houses with a garden, reported nuisance was much higher ($F = 10.45$, $P < 0.03$) and the reported tolerance (N intolerable bites: $F = 0.54$, $P = 0.50$; N tolerable bites: $F = 0.02$, $P = 0.90$) lower compared to people living in a block of flats (Table 7).

The nuisance level reported by families with children <5 years was much higher ($F = 58.03$, $P < 0.002$) compared to other families. However, families with children <5 years did not show differences in reported tolerance (N intolerable bites: $F = 0.07$, $P = 0.80$; N tolerable bites: $F = 0.04$, $P = 0.85$).

Table 6. Stepwise regression analysis of the nuisance levels and data related to captures of indigenous mosquitoes.

		Nuisance					
	Comment	Parameters	SE	t	P	β	SE of β
Intercept		0.765	0.119	6.414	<0.001		
Cx. pipiens	Pooled						
Ae. caspius		0.006	0.001	5.056	<0.001	0.707	0.140
Ceratopogonidae	Pooled						
Eggs/week		0.004	0.001	5.464	<0.001	0.764	0.140

Table 7. Nuisance level and number of bites found as tolerable and intolerable according to different socioeconomic factors analyzed.[1]

			Nuisance		No. bites/day (Average ± SD)			
		N	Average ± SD		Intolerable		Tolerable	
Sex	Women	552	**1.51 ± 1.00 a**	*	371 (5.04 ± 4.22)	NS	369 (3.44 ± 2.95)	NS
	Men	188	**1.28 ± 0.83 b**		135 (5.15 ± 4.70)		135 (3.47 ± 2.60)	
Age	18–29	77	1.56 ± 0.77	NS	68 (5.04 ± 3.10)	NS	66 (3.27 ± 2.10)	NS
	30–44	140	1.44 ± 0.90		112 (5.28 ± 5.11)		109 (3.39 ± 2.37)	
	45–64	275	1.48 ± 0.94		198 (5.24 ± 4.93)		198 (3.54 ± 3.28)	
	>65	230	1.33 ± 0.95		119 (4.59 ± 3.04)		122 (3.38 ± 2.94)	
Dwelling	Block of flats	135	**1.20 ± 0.90 a**	*	84 (4.68 ± 4.02)	NS	81 (3.51 ± 3.87)	NS
	Independent house	601	**1.51 ± 0.97 b**		419 (5.15 ± 4.42)		420 (3.43 ± 2.63)	
Children <5 years	Without children	646	**1.41 ± 0.95 a**	**	437 (5.04 ± 4.53)	NS	434 (3.46 ± 2.94)	NS
	With children	94	**1.74 ± 0.98 b**		69 (5.30 ± 2.95)		70 (3.39 ± 2.29)	
Profession	Housewife	150	1.45 ± 1.01	NS	103 (5.05 ± 3,32)	NS	97 **(3.23 ± 2.71) a**	*
	Teacher	61	1.62 ± 0.92		50 (5.67± 4.43)		51 **(4.60 ± 4.66) b**	
	Retired	236	1.39 ± 1.02		137 (4.41 ± 3.67)		139 **(3.13 ± 2.62) a**	
	Student	56	1.48 ± 0.83		49 (5.19 ± 3.24)		48 **(3.32 ± 2.05) a**	
	Other professions	199	1.42 ± 0.85		147 (5.50 ± 5.75)		149 **(3.49 ± 2.57) a**	
Education	Primary	148	**1.23 ± 1.02 a**	*	72 (5.48 ± 6.19)	NS	68 (3.37 ± 2.43)	NS
	Secondary	197	**1.58 ± 0.97 b**		140 (5.00 ± 5.09)		139 (3.29 ± 2.87)	
	High school	263	**1.51 ± 0.87 b**		206 (4.85 ± 2.99)		209 (3.24 ± 2.37)	
	University	66	**1.36 ± 0.83 ab**		58 (5.52 ± 4.35)		57 (4.54 ± 4.48)	
Domestic tools	None	53	**0.96 ± 1.00 a**	**	16 (6.16 ± 3.21)	NS	19 (5.63 ± 4.76)	NS
	Insecticide	298	**1.50 ± 0.94 b**		218 (5.29 ± 3.52)		217 (3.62 ± 3.02)	
	Net	112	**1.32 ± 1.01 ab**		67 (4.31 ± 3.00)		65 (3.04 ± 2.83)	
	Prevention + insecticide	55	**1.49 ± 0.88 b**		43 (5.45 ± 5.04)		40 (3.38 ± 1.99)	
	Prevention + net	21	**1.76 ± 1.14 b**		18 (4.06 ± 3.21)		18 (2.64 ± 2.39)	
	Net + insecticide	167	**1.50 ± 0.92 b**		116 (4.76 ± 5.06)		113 (3.23 ± 2.50)	
	Prevention + net + insecticide	33	**1.70 ± 0.95 b**		27 (5.93 ± 8.29)		31 (3.10 ± 2.22)	
Gardening	No change of customs	87	**1.28 ± 0.83 a**	**	37 (4.97 ± 2.68)	NS	46 (5.18 ± 3.69)	NS
	Change in the use of green spaces	68	**2.28 ± 0.84 b**		46 (6.74 ± 8.49)		49 (4.56 ± 4.63)	

[1] Significance is indicated with asterisks (*$P < 0.05$; **$P < 0.01$). NS, not significant, bold defines the parameters that are significantly different.

The occupations of participants were grouped in 5 categories: housewives and retired people (citizens spending most of the day at home), teachers and students (professions taking up only a part of the day), and the other professions. We did not observe differences in the nuisance ($F = 1.13$, $P = 0.38$) or reported intolerable level (N intolerable bites: $F = 1.73$, $P = 0.19$), but significant differences were found regarding the number of tolerable bites ($F = 3.34$, $P < 0.04$), with teachers declaring the highest tolerance.

Educational level was related to differences in reported nuisance ($F = 4.42$, $P < 0.03$) but not in the number of tolerable ($F = 2.57$, $P = 0.10$) and intolerable ($F = 0.40$, $P = 0.76$) bites. The interviewed people with a lower educational level (mostly elderly) and the ones with a higher educational level (degree) declared a lower nuisance level compared to respondents with a medium educational level (secondary school).

The protection tools were divided in 7 categories: none (no control or prevention tools used); insecticide (use of home insecticides such as electric mats, mosquito coil, repellents, etc.); net (mosquito nets for windows); net + insecticide (mosquito nets and insecticides); prevention + insecticide (prevention tools such as removal of focus, larval control, etc., and insecticides); prevention + net (prevention tools and mosquito nets); prevention + net + insecticide (prevention tools, mosquito nets, and insecticides). We observed some important differences in mosquito nuisance ($F = 7.58$, $P < 0.002$) according to the tools used (Table 7). The interviewed people who did not use tools declared a definitely lower nuisance compared to the ones using some. We did not observe important differences related to the tolerable and intolerable bites according to the type of control tools used ($F = 1.79$, $P < 0.14$; $F = 1.08$, $P = 0.40$).

Generally, it was observed that in people living in a block of flats usually used mosquito nets for windows (55%) and home insecticides (67%), while more than 11% of the interviewed people declared use of any tool. In independent houses, only 6% of the people interviewed used any tools and the percentage who used insecticides and mosquito nets was 77% and 43%, respectively. Furthermore, 16.5% of families living in a house with garden carried out some prevention activity.

The questionnaire asked whether the use of green spaces was reduced by mosquitoes in these last years (*Ae. albopictus* was found for the first time in 1999 in Rovigo and in 2001 in Villadose). The percentage of interviewed people declaring a reduction in outdoor activities was 54% in Rovigo, the area where *Ae. albopictus* infestation is higher, and 31% in Villadose. Moreover, it was observed that people living in independent houses with garden were more likely to have changed their customs (56% in Rovigo and 36% in Villadose) compared to the ones living in block of flats (36% in Rovigo and 9% in Villadose) (Table 7).

Turning the parameters into dummy variables, we tried to highlight how the ratio between nuisance and mosquitoes varies according to the kind of dwelling and to the presence of children in the family. Considering dwelling as a dummy variable (independent houses and block of flats), the regression analysis points out a good correlation (Table 8). The equation linking the different variables is

$$M_{\text{house}} = 0.51 + 0.33A + 0.0038Ae.c. + 0.0043E_{Ae.a.}\left(R^2 = 0.70, F_{3,16} = 12.20, P < 0.0001\right), \quad (2)$$

where $A = 1$ for detached houses and $A = 0$ for block of flats. In residential areas characterized by independent houses with garden, the equation becomes

$$M_{\text{ind.houses}} = 0.84 + 0.0038Ae.c. + 0.0043E_{Ae.a.}. \quad (3)$$

In areas with blocks of flats, the equation is

$$M_{\text{block}} = 0.51 + 0.0038Ae.c. + 0.0043E_{Ae.a.}. \quad (4)$$

The presence of children seems important for the definition of the families' tolerable levels and sensitivity to nuisance (Table 9). In cases where there are children, sensitivity was definitively higher ($R^2 = 0.71$, $F_{3,16} = 12.94$, $P < 0.001$).

$$M_{\text{children}} = 1.12 + 0.007Ae.c. + 0.0046E_{Ae.a.}, \quad (5)$$

Table 8. Parameter by stepwise regression analysis of the nuisance levels and data related to the captures of native mosquitoes carried out with CO_2 traps and average number of *Ae. albopictus* eggs collected with ovitraps, considering dwelling as the dummy variable.

	Nuisance					
	Parameters	SE	t	P	β	SE of β
Intercept	0.508	0.148	3.427	0.0034		
Dummy	0.332	0.130	2.566	0.0207	0.354	0.138
Ae. caspius	0.004	0.001	2.898	0.0104	0.408	0.141
Eggs/week	0.004	0.001	5.135	0.0001	0.722	0.141

Table 9. Parameter by stepwise regression analysis of the nuisance levels and data related to the captures of native mosquitoes carried out with CO_2-baited traps and mean *Ae. albopictus* eggs picked up with ovitraps, considering presence of children <5 years as the dummy variable.

	Nuisance					
	Parameters	SE	t	P	β	SE of β
Intercept	0.661	0.185	3.566	0.0026		
Dummy	0.461	0.162	2.840	0.0118	0.3836	0.1351
Ae. caspius	0.007	0.002	4.242	0.0006	0.5846	0.1378
Eggs/week	0.005	0.001	4.344	0.0005	0.5987	0.1378

$$M_{\text{no children}} = 0.46 + 0.007Ae.c. + 0.0046E_{Ae.a.}. \quad (6)$$

Lastly, the equation linking the nuisance index M with the percentage of the population considering the trouble as tolerable (P) was calculated:

$$P = -0.4416M + 1.2859 \quad (7)$$
$$\left(R^2 = 0.98, F_{1,8} = 503.43, P < 0.0001\right).$$

Therefore, a threshold $M = 1$ implied that most citizens (84%) found the presence of mosquitoes tolerable; with a threshold $M = 1.5$, we find that 60% of the population consider the level tolerable; with a threshold $M = 2$, the percentage of the population considering mosquitoes tolerable was only 40%.

DISCUSSION

Our observations confirm previous findings regarding the high cost and the low effectiveness of CO_2 traps in monitoring *Ae. albopictus* population density (Kröckel et al. 2006). Data obtained by 8 ovitraps/km^2 were sufficient to evaluate the nuisance level caused by *Ae. albopictus* and showed to be in good agreement with data produced by the container inspections.

According to this study, it is evident that people in this area had a sensitivity level to mosquitoes similar to or lower than that found in other locations (John et al. 1987, Morris and Clanton 1988, Read et al. 1994), with the number of bites considered as intolerable by the interviewed people on average 5/day.

There are social, psychological, and biomedical factors affecting the sensitivity differences observed in the survey. The way green spaces are used, differing according to various socio-demographic factors, can influence the sensitivity to diurnal mosquitoes because it affects the time spent outdoors when the mosquitoes are active. Indeed, the data analysis points out that the presence of children strongly affects the parents' sensitivity.

Physiological factors may also play a relevant role in the sensitivity to mosquito bites. Peng et al. (1996) and Peng and Simons (1998) have observed that there is an inverse correlation of the skin reactions to mosquito bites with the duration of exposure, and have hypothesized that natural exposure could induce desensitization phenomena.

Many citizens use home insecticides (more than 70% of the interviewed people) in addition to mosquito nets for windows (about 50%) in order to protect themselves. While these protection tools seem adequate for the common house mosquitoes *Cx. pipiens* (nocturnal and endophilic), on the contrary, for the more anthrophophilic and exophilic species *Ae. albopictus* and *Ae. caspius*, respectively diurnal and crepuscular, citizens seem to be much more exposed.

In residential areas, *Ae. albopictus* is markedly modifying citizens' behavior in use of green spaces, and more than 50% of the people interviewed in Rovigo declared that mosquitoes limit their outdoor activities. Consequently, in residential environments a decrease in the use of green spaces as a behavioral defense against mosquitoes can happen. Therefore, control measures against diurnal and crepuscular mosquitoes have a strong social value.

We obtained quite a good relationship between the nuisance level declared by residents and the captures obtained with CO_2-baited traps (*Ae. caspius*) and ovitraps (*Ae. albopictus*). An equation with which it was possible to estimate the nuisance level according to the different kind of dwelling and to the presence of children was developed. In residential areas where most of the dwellings are houses with a garden, it is appropriate to use Eq. (3) to estimate the nuisance, while in areas where blocks of flats prevail, it was more appropriate to use Eq. (4). The presence of children determines a meaningful increase of sensitivity; therefore, in schools, in parks, and, generally, in areas used by children, Eq. (5) can be appropriate.

To verify the threshold obtained in this study, Eq. (1) was compared with the equation defined in Piedmont region (PRB 2003). The 2 equations define very similar tolerance thresholds: considering *Ae. caspius* only, when the nuisance index $M = 1$ we have 40 *Aedes* females, while with the Piedmont equation when the tolerance threshold (TT) = 1.6, this gave 39 *Aedes* females (Fig. 2).

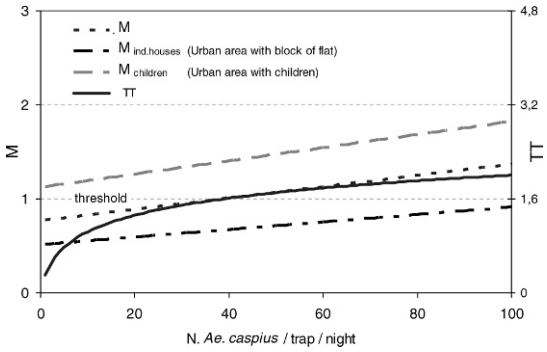

Fig. 2. Comparison between the empirical equation defined according to the experience in Piedmont (threshold of tolerance, TT) and the one defined by this study (M_t, $M_{\text{Ind.House}}$, and M_{children}).

This study defines the tolerable density of mosquitoes below which it was not necessary to increase control activities (nuisance index $M = 1$) and the mosquito density at which it was strongly recommended to increase control efforts because the problem was serious (nuisance index $M = 2$). The tolerance threshold which can be adopted in mosquito control programs is found in this interval ($1 < M < 2$).

An economic assessment of the benefits and costs of mosquito control should be done to establish an economic threshold based on these tolerance levels.

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Centers for Disease Control and Prevention

Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control

U.S. Department of Health and Human Services
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INTRODUCTION

In late summer 1999, the first domestically acquired human cases of West Nile (WN) encephalitis were documented in the U.S.[1-6] The discovery of virus-infected, overwintering mosquitoes during the winter of 1999-2000 presaged renewed virus activity for the following spring and precipitated early season vector control and disease surveillance in New York City (NYC) and the surrounding areas.[7, 8] These surveillance efforts were focused on identifying and documenting WN virus (WNV) infections in birds, mosquitoes and equines as sentinel animals that could alert health officials to the occurrence of human disease. Surveillance tracked the spread of WNV throughout much of the U.S. between 2000 and 2002. By the end of 2002, WNV activity had been identified in 44 states and the District of Columbia. The 2002 WNV epidemic and epizootic resulted in reports of 4,156 reported human cases of WN disease (including 2,942 meningoencephalitis cases and 284 deaths), 16,741 dead birds, 6,604 infected mosquito pools, and 14,571 equine cases. The 2002 WNV epidemic was the largest recognized arboviral meningoencephalitis epidemic in the Western Hemisphere and the largest WN meningoencephalitis epidemic ever recorded. Significant human disease activity was recorded in Canada for the first time, and WNV activity was also documented in the Caribbean basin and Mexico. In 2002, 4 novel routes of WNV transmission to humans were documented for the first time: 1) blood transfusion, 2) organ transplantation, 3) transplacental transfer**,** and 4) breast-feeding.

WNV is a member of the family Flaviviridae (genus Flavivirus). Serologically, it is a member of the Japanese encephalitis virus antigenic complex, which includes St. Louis, Japanese, Kunjin, and Murray Valley encephalitis viruses.[9,10] WNV was first isolated in the WN province of Uganda in 1937.[11,12] Human and equine outbreaks have been recorded in portions of Africa, southern Europe, North America, and Asia.[13,14]

Although it is still not known when or how WNV was introduced into North America, international travel of infected persons to New York, importation of infected birds or mosquitoes, or migration of infected birds are all possibilities. In humans, WNV infection usually produces either asymptomatic infection or mild febrile disease, sometimes accompanied by rash, but it can cause severe and even fatal diseases in a small percentage of patients. The human case-fatality rate in the U.S. has been 7% overall, and among patients with neuroinvasive WNV disease, 10%.

Unlike WNV within its historical geographic range, or St. Louis encephalitis (SLE) virus in the Western Hemisphere, mortality in a wide variety of bird species has been a hallmark of WNV activity in the U.S. The reasons for this are not known; however, public health officials have been able to use bird mortality (particularly birds from the family Corvidae) to effectively track the movement of WNV. WNV has now been shown to affect 162 species of birds. Previous early-season field studies have determined that areas with bird mortality due to WNV infection were experiencing ongoing enzootic transmission. However, most birds survive WNV infection as indicated by the high seroprevalence in numerous species of resident birds within the regions of most intensive virus transmission. The contribution of migrating birds to natural transmission cycles and dispersal of both WN and SLE viruses is poorly understood.

WNV has been transmitted principally by *Culex* species mosquitoes, the usual vectors of SLE virus. Thirty-six species of mosquitoes have been shown to be infected with WNV. This wide variety of WNV-infected mosquito species has widened this virus' host-range in the U.S.: 27 mammalian species have been shown to be susceptible to WNV infection and disease has been reported in 20 of these (including humans and horses). It must be remembered, however, that the detection of WNV in a mosquito species is necessary but not sufficient to implicate that species as a competent vector of WNV.

Since 1999, the Centers for Disease Control and Prevention (CDC) and a variety of other U.S. governmental agencies and partners have sponsored yearly national meetings of arbovirologists, epidemiologists, laboratorians, ecologists, vector-control specialists, wildlife biologists, communication experts, and state and local health and agriculture officials to assess the implications of the WNV introduction into the U.S. and to refine the comprehensive national response plan. Recommendations from these meetings have been used to develop and to update these guidelines.[15,16] This document is available electronically from the CDC Web site at: http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm.

To assist guideline implementation in 2000, CDC developed an electronic-based surveillance and reporting system (ArboNet) to track WNV activity in humans, horses, other mammals, birds and mosquitoes. In 2003, the ArboNet surveillance system has been updated to streamline reporting to CDC of WNV activity by the state public health departments.

Today=s rapid transport of people, animals, and commodities increase the likelihood that other introductions of exotic pathogens will occur. CDC continues to implement its plan titled APreventing Emerging Infectious Diseases, a Plan for the 21st Century".[17]

I. SURVEILLANCE

A universally applicable arbovirus surveillance system does not exist. In any given jurisdiction, surveillance systems should be tailored according to the probability of arbovirus activity and available resources. In jurisdictions without pre-existing vector-borne disease surveillance and control programs, newly developed avian-based and/or mosquito-based arbovirus surveillance systems will be required. In some, resurrection of previously abandoned systems will be necessary. In others, modification and/or strengthening of existing arbovirus surveillance systems (i.e., those intended to monitor eastern equine encephalitis [EEE], western equine encephalitis [WEE], and/or St. Louis encephalitis [SLE] virus activity) will be the most appropriate response. In yet other jurisdictions in which the probability of arbovirus activity is very low and/or resources to support avian-based and/or mosquito-based surveillance are unavailable, laboratory-based surveillance for neurologic disease in humans and equines should be employed at minimum.

Seasonality of surveillance activities may vary depending upon geographic region. With the anticipated spread of West Nile virus (WNV) to all of the 48 contiguous United States in 2003, all states should initiate surveillance after mosquitoes become active in the spring.

Appropriate and timely response to surveillance data is the key to preventing human and animal disease associated with WNV and other arboviruses. That response must include effective mosquito control and public education without delay, if an increasing intensity of virus activity is detected by bird- or mosquito-based surveillance systems (see Section III.M). For basic information on arbovirus surveillance, see CDC Guidelines for Arbovirus Surveillance Programs in the United States,[18] this document can be obtained from CDC's Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado, and is also available from the CDC Web site at: www.cdc.gov/ncidod/dvbid/arbor/arboguid.htm.

A. Ecologic Surveillance

Detection of WNV in bird and mosquito populations helps health officials predict and prevent human and domestic animal infections. Surveillance to detect WNV should focus on the avian and mosquito components of the enzootic transmission cycle. Non-human mammals, particularly equines, may also serve as effective sentinels because a high intensity of mosquito exposure makes them more likely to be infected than people. Descriptions of the avian-, mosquito-, and non human mammal-based surveillance strategies follow.

1. Avian

a) Avian morbidity/mortality surveillance

Avian morbidity/mortality surveillance appears to be the most sensitive early detection system for WNV activity, and should be a component of every state's arbovirus surveillance program. Its utility for monitoring ongoing transmission in a standardized fashion is currently being investigated, but should include at least two basic elements: the timely reporting and analysis of dead bird sightings and the submission of selected individual birds for WNV testing.

GOAL OF AVIAN MORBIDITY/MORTALITY SURVEILLANCE: Utilize bird mortality associated with WNV infection as a means of detecting WNV activity in a location.

1) Protocols and specimens

The level of effort involved in this surveillance activity will depend on a risk assessment in each jurisdiction. Generally, avian surveillance should be initiated when local adult mosquito activity begins in the spring. A database should be established to record and analyze dead bird sightings with the following suggested data: caller identification and call-back number, date observed, location geocoded to the highest feasible resolution, species, and condition. Samples from birds in good condition (unscavenged and without obvious decomposition or maggot infestation) may be submitted for laboratory testing. As with all dead animals, carcasses should be handled carefully, avoiding direct contact with skin. For greatest sensitivity, a variety of bird species should be tested, but corvids should be emphasized.[19] The number of bird specimens tested will be dependent upon resources and whether WNV-infected birds have been found in the area; triage of specimens may be necessary on the basis of sensitive species (such as corvids) and geographic location. Many jurisdictions may limit (or even stop) avian mortality surveillance once WNV is confirmed in their region. It is suggested that avian mortality surveillance be continued in each region as long as it remains necessary to know whether local transmission persists, because dead-bird-based surveillance is the most sensitive method for detection of WNV activity in most regions.

A single organ specimen from each bird is sufficient to detect WNV or viral RNA. Kidneys, brains, or hearts are preferable.[20-22] Oral swabs from corvids have been validated as a sensitive alternative to organ samples, and because fewer resources are necessary to acquire them, oral swabs are the preferred specimen from corvid carcasses.[23] Testing involves isolation of infectious virus, specific RNA detection by reverse transcription-polymerase chain reaction (RT-PCR), [24] or antigen detection,[25,26] and will generally be positive within 1-2 weeks after specimen submission.

2) Recent experience

Analysis of recent avian morbidity and mortality data indicated that

(a) The American crow was the most sensitive species for avian morbidity/ mortality surveillance in northern regions. However, some areas did not have WNV-positive American crows, but only WNV-positive birds of other species. In southern regions, blue jays have been more sensitive than crows.
(b) Almost all of the positive birds were found singly and not as part of a mass die-off at a single time and place.
(c) Approximately one-third of the WNV-positive birds had signs of trauma on necropsy.
(d) Many WNV-positive birds did not have pathology indicative of WNV infection on necropsy. No lesions are pathognomonic for WNV infection.
(e) WNV-positive dead birds usually provided the earliest indication of viral activity in an area. In 2002, the detection of WNV-infected dead birds was

the first positive surveillance event in 1,534 (61%) of 2,531 counties reporting WNV activity.

(f) The detection of WNV-positive dead birds preceded reports of human cases (although knowledge of the test result did not necessarily predate the onset of human cases). In 2002, 527 (89%) of 589 counties reporting human WN meningoencephalitis cases first detected WNV transmission in animals. In 327 (72%) of these 527 counties, detection of WNV-infected dead birds was the first positive surveillance event, preceding human illness onset by a median of 38.5 days (range, 2-252 days).

(g) Many counties with human cases of WNV infection tended to have high dead bird surveillance indices, both WNV-positive and sightings. Notable exceptions included sparsely populated counties, particularly those in the midwestern states.[27,28]

(h) Experimental evidence of direct transmission among corvids and gulls exists, but whether this occurs in nature is unknown.[29] If it does, then in some settings, virus-infected mosquitoes might not be necessary to maintain enzootic transmission cycles.

3) *Advantages of avian morbidity/mortality surveillance include the following:*

(a) Certain species of birds, in particular corvids (e.g., crows and jays) experience high clinical attack rates.

(b) The size and coloration of certain dead birds makes them conspicuous (e.g., crows).

(c) RT-PCR and antigen-detection assays can be used to rapidly detect WN viral RNA and protein, respectively, in tissues, even if the tissue is partly decomposed. Both assays have now been adapted for field applications.

(d) Due to public involvement in reporting dead bird sightings, dead wild birds are readily available over a much wider region than can be sampled by other surveillance methods.

(e) Detection of WNV in dead birds likely signifies local transmission.[30]

(f) This type of surveillance provides a temporally and spatially sensitive method for the detection of WNV activity.

(g) It can be used for early detection and possibly also for ongoing monitoring of WNV transmission.

(h) It may be used to estimate risk of human infection with WNV.[27,31,32]

4) *Disadvantages of avian morbidity/mortality surveillance include the following:*

(a) Dead bird surveillance data from different jurisdictions are difficult to compare.

(b) Birds are highly mobile and often have extensive home ranges, so that the site of death may be distant from the site of infection (especially after the breeding season, when birds are generally less territorial).

(c) Collection, handling, shipping, and processing of birds or their clinical specimens is cumbersome.

(d) Systems for handling, processing, and testing have at times been overwhelmed by high public response and public expectations.

(e) The long-term usefulness of this system is uncertain because natural selection for disease-resistant birds may occur, populations of susceptible species may become very low, or the virus may evolve, resulting in low or no

avian mortality. In areas where WNV annually recurs, intense environmental sampling might not be as useful.

(f) Success is influenced by public participation, which is highly variable, and depends on the number of public outreach programs, level of public concern, etc.

(g) The system may be less sensitive in rural areas, where there are fewer persons to observe dead birds over a wider geographic area. In the western U.S., low observer density is coupled with the presence of a vector (*Culex tarsalis*) that is less ornithophilic, resulting in fewer reports of dead birds relative to other non-avian surveillance indicators.

b) Live bird surveillance

Live-bird surveillance has been used traditionally both to detect and monitor arbovirus transmission (e.g., for SLE, EEE and WEE viruses). Two approaches are captive sentinel surveillance (typically using chickens, but other species have been used as well), and free-ranging bird surveillance.[33] Both depend on serological testing, which generally requires at least 3 weeks to detect and confirm an infection. Successful application of these approaches requires extensive knowledge of local transmission dynamics. It is recommended that further research be done before relying on sentinel birds as a primary means of WNV surveillance. Use of sentinel birds may require institutional animal use and care protocols, and other authorization permits.

GOAL OF LIVE-BIRD SURVEILLANCE: Utilize seroconversions in captive or free-ranging bird species as indicators of local WNV activity.

1) Captive sentinel surveillance

Although an ideal captive avian sentinel for WNV -- or any other arbovirus – may not exist, such a species would meet the following criteria: 1) is universally susceptible to infection, 2) has a 100% survival rate from infection and universally develops easily detectable antibodies, 3) poses no risk of infection to handlers, and 4) never develops viremia sufficient to infect vector mosquitoes.[18] Captive sentinels have been effectively used to monitor transmission of arboviruses in a standardized fashion, including SLE virus in California and Florida, especially in historical enzootic transmission foci. Captive sentinel flocks should be placed in likely transmission foci (e.g., near vector breeding sites or adult mosquito congregation sites), and presented appropriately to allow feeding by enzootic WNV vectors. Alternatively, pre-existing captive birds (e.g., domestic poultry or pigeons, or zoo birds) may be used as sentinels.

(a) Protocols and specimens
Whole blood can be collected and centrifuged for serum. Serum is screened by either hemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA) or plaque-reduction neutralization test (PRNT).[34] It is important to note that the extraction of avian serum samples to remove non specific inhibitors of hemagglutination for use in the HI test follows procedures different from those used in tests of

human serum samples.[35] Positive tests must be confirmed by neutralization to rule out false positives and cross-reactions due to infection with related flaviviruses (e.g., SLE virus).

(b) Recent experience
   (i) In 2000, sentinel chickens were used in selected counties in New York State, New York City (NYC), New Jersey, Pennsylvania, Maryland, and Delaware. Small numbers of seroconversions were detected late in the season in New Jersey and New York. As used in 2000, chickens were ineffective sentinels. In NYC in 2001, sentinel chickens were placed in known transmission foci and seroconverted earlier in the season, but not earlier than the first human cases. In 2002, hundreds of sentinel chickens in the Southeast seroconverted, but these were rarely the earliest indicators of WNV activity at the county level.
   (ii) IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) testing of experimentally infected chickens points to the need for biweekly sampling of sentinels.[36]
   (iii) Experimental studies have shown that chickens, pigeons, and pheasants (CDC, unpublished data) are candidate sentinels due to their susceptibility to infection, low mortality, and relative incompetence as amplifying hosts. However, small amounts of WNV were detected in cloacal swabs from infected chickens and pigeons.[29,37]
   (iv) Field studies of avian seroprevalence in Queens in 1999 indicated that captive chickens frequently were infected.[38] In Staten Island in 2000, captive pigeons frequently were infected.[39]
   (v) Some mortality in chickens was attributed to WNV at various locations in New York State.[40]

(c) Advantages of sentinel captive bird surveillance include the following:
   (i) Chickens have been successfully used in flavivirus surveillance for over 6 decades.
   (ii) Birds are readily fed upon by *Culex* mosquitoes.
   (iii) Captive birds can be serially bled, making the geographic location of infection definite.
   (iv) The system is flexible and therefore can be expanded and contracted as appropriate.
   (v) Mosquito-abatement districts can maintain and bleed flocks and submit specimens for testing.
   (vi) Collection of specimens is inexpensive compared with the costs of free-ranging bird surveillance.

(d) Disadvantages of captive sentinel surveillance include the following:
   (i) Sentinel flocks detect only focal transmission, requiring multiple flocks be positioned in representative geographic areas. This is particularly true when vector mosquitoes have short flight ranges (e.g., *Culex pipiens*).
   (ii) Flocks are subject to vandalism and theft.
   (iii) Flocks must be protected from predators.

(iv) Flock set-up and maintenance (i.e., birds, cages, feed, transportation) are expensive. Training is required for proper maintenance and sampling.
(v) Pre-existing flocks may already have been exposed due to previous local WNV transmission.

2) *Free-ranging bird surveillance*

Free-ranging birds provide the opportunity for sampling important reservoir host species and may be used both for early detection and for monitoring virus activity. This type of surveillance has been used effectively for SLE, EEE and WEE virus surveillance in several states. In each geographic area, the optimal free-ranging bird species to be monitored should be determined by serosurveys. The best species for serologic surveillance are those in which infection is rarely, if ever, fatal, and population replacement rates are high, ensuring a high proportion of uninfected individuals.

(a) Protocols and specimens
The use of free-ranging birds requires differentiation of recent infection from infections acquired in previous years. For most species, assays for detection of IgM antibody will not be available and other tests such as IgG (IgY)-detection ELISAs[41,42] and the PRNT[34] must be used to detect WNV-specific antibody. Antibody-positive birds less than 1 year old may be presumed to have been infected recently (during current transmission season). Weak seropositivity in very young birds (less than 1 month old) may be due to maternal transfer of antibody. Seroconversion in older birds is also evidence of recent transmission, but requires frequent recapture for acquisition of multiple specimens from uniquely banded individuals during the course of the transmission season. WNV seropositivity among after-hatch-year birds, when determined from a single serum specimen, should not be interpreted or reported as evidence of recent infection. State and federal permits are required for capture and banding of federally-protected migratory birds.

(b) Recent experience
(i) In urban epizootic transmission foci in NYC, several common species (i.e., house sparrows, cardinals, catbirds, mourning doves, rock doves) developed high seroprevalence, making them strong candidate sentinels, although other species may be important in other locations.[38,39]
(ii) A comparison of free-ranging bird surveillance in NYC in 2001 found that much greater effort was required for this surveillance system compared with other surveillance systems (Green Street Scientific, LLC, unpublished data). Similar observations have been made in Indiana, Louisiana, New Jersey, Ohio, and Texas.

(c) Advantages of free-ranging bird surveillance include the following:
(i) It has a long history of successful use in flavivirus surveillance.
(ii) Local movement of resident wild birds may increase contact with enzootic transmission foci, thus increasing sensitivity (relative to captive sentinels).
(iii) Set-up or maintenance costs may be minimal.

(iv) Its sampling capability is highly flexible.
(v) It permits evaluation of herd immunity among important amplifying hosts.
(vi) Owner confidentiality may be less of an issue.

(d) Disadvantages of free-ranging bird surveillance include the following:
(i) Interpretation of serologic results is complex.
(ii) Handling and venipuncture of birds increases the risk of exposure to pathogens in blood and feces.
(iii) Movement of free-ranging wild birds makes it impossible to know where an infection was acquired.
(iv) Most birds are protected by federal law, and their collection and sampling requires state and federal permits. Banding permits require complex data reporting.
(v) Training is required for live-trapping, blood-sampling, handling, and accurate determination of the species and age of wild birds.
(vi) It is generally not feasible to serially bleed individual free-ranging birds because of low recapture rates (although banding can be useful).

2. Equine

Equines appear to be important sentinels of WNV epizootic activity and human risk, at least in some geographic regions. In addition, equine health is an important economic issue. Therefore, surveillance for equine WNV disease should be conducted in jurisdictions where equines are present. Veterinarians, veterinary service societies/agencies, and state agriculture departments are essential partners in any surveillance activities involving equine WNV disease. A working surveillance case definition of clinical WNV infection in equines is presented in Appendix B.

GOALS OF EQUINE DISEASE SURVEILLANCE: To use data on equine WNV disease cases to assess the threat of human disease, identify geographic areas of high risk, and assess the need for and timing of interventions.

a) Protocols and Specimens

1) Serum and cerebrospinal fluid (CSF) for antibody testing. Because an equine WNV vaccine is now in widespread use, a complete vaccination history should accompany all specimens submitted for antibody testing.

2) Necropsy tissues (especially brain and spinal cord) for gross pathology, histopathology, RT-PCR, virus isolation, and immunohistochemistry. The differential diagnosis of equine encephalitis includes, but is not limited to, the other arboviral encephalitides and rabies.

b) Recent experience

1) In 2002, equine WNV disease cases were the first indication of WNV activity in 95 (16%) of the 589 counties where human disease was reported. The majority of these 95 counties were located in the central and western U.S.

2) In general, equine WNV disease cases have been scattered. Few case clusters have been documented.

3) In fatal equine WNV disease cases, pathological findings have been non-specific. Pathognomonic lesions have not been described.

4) A licensed equine WNV vaccine has been available in the U.S. since 2001. No studies of efficacy have been published.

c) Advantages of equine disease surveillance include the following:

1) Equines are highly conspicuous, numerous, and widely distributed in some areas. They may be particularly useful sentinels in rural areas, where dead birds may be less likely to be detected.

2) Some equines are routinely bled and tested for other pathogens.

3) Ill equines have been one of the earliest, if not the earliest, sentinels of WNV activity in some geographic areas.

d) Disadvantages of equine disease surveillance include the following:

1) In some geographic areas, equines may not be an early sentinel (i.e., human WNV disease cases may occur simultaneously with or soon after equine cases).

2) Necropsies are expensive and logistically difficult.

3) Equines are not present or abundant in many areas of the U.S. (e.g., densely populated metropolitan areas), and proximity of equines to human populations varies.

4) Widespread use of equine WNV vaccines may decrease the incidence of equine WNV disease and therefore the usefulness of equines as sentinels.

5) Because the costs of clinical equine specimen collection and testing are usually borne directly by the owner, economic factors work against the submission and testing of equine specimens for arboviral infections.

e) Minimal components of an equine surveillance program

1) All equine neurologic disease cases should be promptly reported; the equines should be tested for infection with WNV and other arboviruses as geographically appropriate, and for rabies.

2) Clusters of equine neurologic disease cases should be promptly investigated.

3. Mosquito

While dead-bird-based surveillance has proven to be the most sensitive method of detecting WNV presence in an area, mosquito-based surveillance remains the primary tool for quantifying the intensity of virus transmission in an area, and should be a mainstay in most surveillance programs for WNV and other arboviruses.

GOALS OF MOSQUITO-BASED SURVEILLANCE: To 1) use data on mosquito populations and virus infection rates to assess the threat of human disease; 2) identify geographic areas of high risk; 3) assess the need for and timing of interventions; 4) identify larval habitats for targeted control; 5) monitor the effectiveness of this type of surveillance and improve prevention and control measures; and 6) develop a better understanding of transmission cycles and potential vector species.

a) Protocols and specimens

1) Adult mosquitoes are collected using a variety of trapping techniques and are used to identify the mosquito species and primary vector species present in an area and the relative density of those species. When coupled with virus detection protocols, mosquito collections can be screened for the presence of virus and provide a quantifiable index of WNV activity. Adequate sampling requires trapping regularly at representative sites throughout a community, and rapid testing of collections of sufficient size to detect low infection rates in the vector population. Minimally, adult mosquito density (number collected per trap night) and infection rate (number of individual mosquitoes estimated containing WNV per 1,000 specimens tested) should be recorded for each area to provide a basis for tracking mosquito density and virus incidence.

2) Larval mosquitoes are collected by taking dip samples from a variety of habitats to identify species present in the area and to identify mosquito sources. Thorough mapping of larval habitats will facilitate larval control or source reduction activities. In addition, where larval management is not feasible, quantitative estimates of larval densities will permit anticipation of new adult emergences. Minimally, the number of larvae collected per dip and location where collected should be recorded to provide a basis for tracking larval production and association of larval density with resulting adult mosquito population density.

b) Recent experience

1) If mosquito trapping effort is intensive, detection of WNV in mosquitoes might precede detection of virus activity by other surveillance tools. If mosquito trapping effort is inadequate, WNV-positive mosquitoes may not be detected prior to the identification of a virus in dead bird, sentinel animal, or human WNV disease cases.

2) Moderate to high infection rates sustained for several weeks in *Cx. pipiens* or *Cx. quinquefasciatus* have been associated with subsequent human outbreaks. Sustained high infection rates early in the year are associated with a higher risk for subsequent outbreaks.

3) Several intense, focal outbreaks during 2002 were associated with relatively low vector densities, but with high infection rates in key vector species (i.e., infection rates in *Cx. pipiens* or *Cx. quinquefasciatus* of approximately 10 per 1,000 or greater).

4) Large numbers of WNV-positive *Cx. tarsalis* pools have been found in association with WNV activity in areas where this species is common. Meaningful infection rates have not yet been determined.

5) Avian epizootics may occur without demonstrable human WNV infection. The epizootics are demonstrated, in part, by detection of WNV-positive mosquito pools containing only species that feed predominantly on birds (e.g., *Cx. restuans*).

6) During 1999-2002, WNV was detected in 36 mosquito species in the U.S. (see www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm). The vast majority of isolates came from *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. restuans*. Numerous isolates have also come from several potential accessory vectors (i.e., *Cx. tarsalis, Cx. salinarius, Oc. Ae. albopictus, Oc. triseriatus, Ae. vexans, Cx. nigripalpus*). While detection of WNV in these species demonstrates intensified virus transmission (i.e., virus in primarily mammal-feeding or opportunistic mosquitoes), the contribution of these species to human risk is poorly understood.

c) Advantages of mosquito-based surveillance include the following:

1) It may provide the earliest evidence of transmission in an area.

2) It helps establish information on potential mosquito vector species.

3) It provides an estimate of vector species abundance.

4) It gives quantifiable information on virus infection rates in different mosquito species.

5) It provides quantifiable information on potential risk to humans and animals.

6) It provides baseline data that can be used to guide emergency control operations.

7) It allows evaluation of control methods.

d) Disadvantages of mosquito-based surveillance include the following:

1) It is labor-intensive and expensive.

2) Substantial expertise is required for collecting, handling, sorting, species identification, processing, and testing.

3) Collectors may be at risk from mosquito bites, especially if day biting species are important bridge vectors, and should wear topical repellents and/or repellent-treated clothing when working in areas where a risk of WNV transmission exists.

e) Minimal components of an entomological surveillance program

A comprehensive mosquito surveillance program must include larval and adult sampling components, a mapping/record keeping component, a virus-testing component, and a data analysis component. To provide useful data, the surveillance program must be sustained and maintain a consistent effort over several seasons. The exact design of mosquito-based surveillance programs will vary by geography and availability of financial and personnel resources. Not every community will be able to support a comprehensive mosquito-based surveillance program. Minimally, a mosquito-based WNV surveillance program must include the following:

1) Collection of adult mosquitoes using gravid traps and/or light traps, providing representative geographic coverage and with sufficient trap sites and trapping frequency to obtain sample sizes required to detect WNV at relatively low infection rates. Use both fixed and flexible trap positions if possible.

(a) Fixed positions allow for the development of a database that would let public health officials compare population data to previous years and spatially map changes in mosquito abundance.
(b) Flexible sites allow for response to epidemiological and natural events (e.g., a suspected human case, dead crow, or a flood).
(c) A variety of trapping methods should be used, including the following:
  (i) CDC light traps baited with CO_2 for sampling potential accessory vectors.
  (ii) Gravid traps for *Cx. pipiens* and *Cx. quinquefasciatus* to sample primary WNV vectors.

(d) Trap distribution will be influenced by the following species factors:
  (i) Habitat diversity, size, and abundance;
  (ii) Resource availability;
  (iii) Proximity to human population centers and/or recreational areas; and
  (iv) Flight range of vector species in the area.

2) Laboratory support to identify the mosquitoes' species, and to test the specimens for the presence of WNV. Determine infection rates by species.

(a) Make arrangements with a lab for testing. Rapid turnaround is essential.
(b) Focus initially on *Culex* mosquitoes to provide first indication of WNV presence.
(c) Once virus is detected in *Culex* mosquitoes, pool and test all potential vector species with emphasis on incriminated or suspected species.

(3) Data management and analysis capabilities to allow tracking of adult mosquito densities and infection rates over time and space. Patterns of virus activity are more likely to be useful than predetermined threshold levels.

(4) Development of a plan with descriptions of actions that will be taken in response to indicators of WNV activity.

B. Surveillance for Human Cases

Because the primary public health objective of surveillance systems for neurotropic arboviruses is prevention of human infections and disease, human case surveillance alone should not be used for the detection of arbovirus activity, except in jurisdictions where arbovirus activity is rare, or resources to support avian-based and/or mosquito-based arbovirus surveillance are unavailable.

GOALS OF SURVEILLANCE FOR HUMAN CASES: To 1) assess the local, state and national public health impact of WNV disease and monitor national trends; 2) demonstrate the need for public health intervention programs; 3) allocate resources; 4) identify risk factors for infection and determine high-risk populations; 5) identify geographic areas in need of targeted interventions; and 6) identify geographic areas in which it may be appropriate to conduct analytic studies of important public health issues.

1. Recent Experience

a) In the U.S. during 1999-2002, the peak human risk for WN viral infection occurred in August and September, although in 2002 human illness onset was reported as early as mid-May and as late as mid-December. In many regions, the peak minimum infection rates in mosquitoes and a rapid increase in the number of reported avian and equine WN viral infections occurred just prior to the period of maximal human risk.

b) In 1999-2002, the majority of reported, confirmed, or probable cases of human WN viral disease were among persons with meningoencephalitis. Testing of patients with aseptic meningitis or unexplained febrile illnesses for evidence of WN viral infection may be beneficial, but can also overwhelm laboratory testing capacity and appears to be of relatively low yield for surveillance purposes since the majority of these cases will not be due to WNV infection.

c) Most patients with WN encephalitis or meningitis (WNME) are older adults, generally over 50 years old. In the U.S. in 1999-2001, the median age among the 142 reported WNME cases was 68 years. In 2002, among 2,942 reported cases of WN meningoencephalitis, the median age was 59 years. Although 21% of reported cases were in persons younger than 40, only 4% of reported cases were in persons younger than 18.

d) When WN viral infections were first identified in the U.S., WN encephalitis was associated with a Guillain-Barrè-like syndrome with generalized muscle weakness. In 1999-2000, generalized muscle weakness was reported in 29% of WN encephalitis cases. In 2002, at least 2 new neurologic syndromes associated with WN viral infection were identified: acute flaccid paralysis (“WN poliomyelitis-like syndrome”) and brachial plexopathy.

e) Using CDC-recommended test methods in public health laboratories, WNV-specific IgM antibody was detected in acute-phase (i.e., those collected 8 or less days after illness onset) serum or CSF specimens, or both, in the large majority of confirmed cases. In contrast, only a small minority of suspected cases were subsequently confirmed in which specific IgM antibody reactivity in acute-phase serum or CSF was in the equivocal or low-positive range.

f) Longitudinal studies of WNME cases have shown that WNV-specific IgM antibody can persist in serum for 12 months or longer.[43] Thus, the presence of WNV-specific IgM antibody in a single serum sample is not necessarily diagnostic of *acute* WN viral infection. For this reason, especially in areas where WNV is known to have circulated previously, suspected, acute WN viral disease cases should be confirmed by observing a fourfold or more change in titer of WNV-specific antibody in serum and the presence of WNV-specific IgM antibody in CSF, when available.

g) In 1999 in the U.S., the sensitivity of polymerase chain reaction (PCR) tests of CSF for the diagnosis of human WN encephalitis cases was only 57%; more recent statistics are currently unavailable. Thus, PCR for the diagnosis of WN viral infections of the human central nervous system (CNS) continues to be experimental and should not replace tests for the detection of WNV-specific antibody in CSF and serum, tests that are far more sensitive.

h) During 1999-2001, 7 cases of uncomplicated WN fever (WNF) were reported in the U.S., which represents 5% of the total number of WNV disease cases reported. In 2002, over 1,100 WNF cases were reported (30% of total). Contributing factors likely include the intensive media attention paid to the 2002 epidemic that may have led to increased consumer demand for WNV diagnostic testing by patients and physicians, and the greater availability of commercial testing. Nevertheless, during 1999-2002, WNF was probably significantly underdiagnosed in the U.S. It has been estimated that approximately 20 WNF cases occur for every WNME case.[44]

i) For suspected WNV disease cases in immunocompromised patients, WNV-specific antibody may not be present. Since longer viremias may be observed in these patients, testing serum and CSF samples for the presence of virus or viral RNA may be useful.

2. Types of Surveillance

a) Clinical syndromes to monitor

Monitoring of encephalitis cases is the highest priority. Monitoring milder illnesses (e.g., aseptic meningitis, Guillain-Barré syndrome, acute flaccid paralysis, and brachial plexopathy, and fever or rash illnesses) is resource-dependent and should be of lower priority.

b) Types of human surveillance

1) Enhanced passive surveillance

In the absence of known WNV activity in an area, enhanced passive surveillance* for hospitalized cases of encephalitis (and milder clinical syndromes as resources allow**), and for patients who have IgM antibodies to

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*Passive surveillance enhanced by general alerts to key health care personnel such as primary care providers, infectious disease physicians, neurologists, hospital infection control personnel, and diagnostic laboratories.

** While human infections with neurotropic arboviruses are usually clinically inapparent, most clinically apparent infections are associated with fever, with or without neurologic manifestations, which can range from

either WN or SLE virus in tests conducted in diagnostic or reference laboratories, should be employed. A high clinical suspicion for arboviral encephalitis should be encouraged among health care providers. When the diagnosis is in doubt, appropriate clinical specimens should be submitted to CDC or another laboratory capable of performing reliable serologic testing for antibodies to domestic arboviruses. Testing of CSF and paired acute- and convalescent-phase serum samples should be strongly encouraged to maximize the accuracy of serologic results.

2) *Active surveillance*

Active surveillance should be strongly considered in areas with known WNV activity. In general, one or both of the following approaches should be taken:
(a) Contact physicians in appropriate specialties (i.e., infectious diseases, neurology, and critical care) and hospital infection control personnel on a regular basis to inquire about patients with potential arboviral infections;
(b) Implement laboratory-based surveillance to identify CSF specimens meeting sensitive but nonspecific criteria for arboviral infections (e.g., mild to moderate pleocytosis and negative tests for the presence of nonarboviral agents such as bacteria, fungi, herpesviruses, and enteroviruses) and test them for evidence of WNV infection.

3) *Special surveillance projects*

Special projects may be used to enhance arboviral disease surveillance. Such projects include the Emerging Infections Network of the Infectious Diseases Society of America (IDSA EIN), Emergency Department Sentinel Network for Emerging Infections (EMERGEncy ID NET), Unexplained Deaths and Critical Illnesses Surveillance of the Emerging Infections Programs (EIP), and the Global Emerging Infections Sentinel Network of the International Society of Travel Medicine (GeoSentinel). In some areas, syndromic surveillance systems may be considered. "Piggy-backing" surveillance for WNME and milder clinical forms of WN viral infection, such as fever with rash or lymphadenopathy, onto existing syndromic surveillance systems, especially those involving large health maintenance organizations, may be considered. Real-time computerized syndromic surveillance in emergency departments, and special surveillance projects to identify WNV disease in pediatric populations, may be useful.

3. Specimens

a) <u>Cerebrospinal fluid (CSF)</u>

In WNME cases, WNV-specific IgM antibody commonly can be found in CSF on the day of illness onset using antibody-capture ELISA. Virus also may be isolated (rarely) or detected by RT-PCR (in up to 60% of cases) in acute-phase CSF samples.

b) <u>Serum</u>

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mild aseptic meningitis to fulminant and fatal encephalitis. Signs and symptoms may include fever, headache, stiff neck, confusion or other mental status changes, nausea, vomiting, meningismus, cranial nerve abnormalities, paresis or paralysis, sensory deficits, altered reflexes, abnormal movements, convulsions, and coma of varying severity. Arboviral meningitis or encephalitis cannot reliably be clinically distinguished from other central nervous system infections.

Paired acute-phase (collected 0-8 days after onset of illness) and convalescent-phase (collected 14-21 days after the acute specimen) serum specimens are useful for demonstration of seroconversion to WNV and other arboviruses by ELISA or neutralization tests. Although tests of a single acute-phase serum specimen may provide evidence of a recent WNV infection, a negative acute-phase specimen is inadequate for ruling out such an infection, underscoring the importance of collecting paired samples. As mentioned previously, antibody synthesis in immunocompromised individuals might be delayed or absent altogether.

c) Tissues

When arboviral encephalitis is suspected in a patient who undergoes a brain biopsy or who dies, tissues (especially brain samples, including samples of cortex, midbrain, and brainstem), heart/venous blood, and buffy coat samples should be submitted to CDC or other specialized laboratories for arbovirus and other testing. Tissue specimens should be divided; half should be frozen at -70°C and the other half fixed in formalin. Available studies include gross pathology, histopathology, RT-PCR tests, virus isolation, and immunohistochemistry.

4. Surveillance Case Definition

The national case definition for arboviral encephalitis (available at www.cdc.gov/epo/dphsi/casedef/encephalitiscurrent.htm) should be used to classify cases as confirmed or probable, once appropriate laboratory results are available (also see Section II). In CDC publications of national arbovirus surveillance data, no distinction is usually made between confirmed and probable human cases for the purposes of case counting.

5. Minimal Components of a Human Surveillance System

Enhanced passive surveillance for hospitalized encephalitis cases of unknown etiology, and for patients who have IgM antibodies to either WN or SLE virus in tests conducted in diagnostic or reference laboratories.

C. Geography and Timing

In general, the WNV transmission season in the U.S. is longer than that for other domestic arboviruses and requires longer periods of ecologic and human surveillance.

1. Northeastern and Midwestern U.S.

In the northeastern states in 2001-2002, human illness onset occurred as early as early July and as late as mid-November. During these same years, avian cases occurred as early as the first week of April and as late as the second week of December. Active ecological surveillance and enhanced passive surveillance for human cases should begin in early spring and continue through the fall until mosquito activity ceases because of cold weather. Surveillance in urban and suburban areas should be emphasized.

2. Southern U.S.

In 2001-2002, WNV circulated throughout the year, especially in the Gulf states. Although, in 2001-2002, human illness onset was reported as early as mid-May and June and as late as mid-December, equine and avian infections were reported in all

months of the year. Active ecologic surveillance and enhanced passive surveillance for human cases should be conducted year round in these areas.

3. Western U.S.

In 2002, WNV activity was first reported among humans and animals in Rocky Mountain states and among animals in Pacific coast states. These events occurred relatively late in the year (mid-August). Predicting the temporal characteristics of future WNV transmission seasons based on these limited reports is not possible. Despite this limitation, active ecological surveillance and enhanced passive surveillance for human cases beginning in early spring and continuing through the fall until mosquito activity ceases because of cold weather should be encouraged.

4. Other Areas of the Western Hemisphere

In 2002, Canada experienced a WNV epidemic in Ontario and Quebec provinces and an equine/avian epizootic that extended from the maritime provinces to Saskatchewan.

Recent serologic evidence supports the conclusion that WNV has now reached Central America. Further spread to South America by migratory birds seems inevitable, if this has not already occurred. Development of surveillance systems capable of detecting WNV activity should be encouraged in the Caribbean and Central and South America. WNV surveillance should be integrated with dengue surveillance in these areas, and with yellow fever surveillance in areas where urban or peri-urban transmission of this virus occurs.

II. LABORATORY DIAGNOSIS

The clinical presentation of most patients with viral encephalitis is similar regardless of the cause. Also, infection by many of the arboviruses that cause encephalitis, including West Nile and St. Louis encephalitis viruses, usually is clinically inapparent, or causes a nonspecific viral syndrome in most patients. Definitive diagnosis, therefore, can only be made by laboratory testing using specific reagents. To be successful, active surveillance must have adequate laboratory support.

The basic laboratory diagnostic tests—and how they should be used at the national, state, and local level—are outlined below. The initial designation of reference and regional laboratories that can do all testing will be based on the availability of biosafety level 3 (BSL3) containment facilities. Details of the surveillance case definition for human West Nile virus (WNV) disease and of how the laboratory diagnostic tests are used to support surveillance are presented in Appendix B.

A. Biocontainment

1. Laboratory Safety Issues

Laboratory-associated infections with WNV have been reported in the literature. The Subcommittee on Arbovirus Laboratory Safety (SALS) in 1980, reported 15 human infections from laboratory accidents. One of these infections was attributed to aerosol exposure. Recently, two parenteral inoculations have been reported during work with animals.

a) WNV may be present in blood, serum, tissues and CSF of infected humans, birds, mammals and reptiles. The virus has been found in the oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals may be at high risk of infection.

b) Biosafety Level 2 practices and facilities are recommended for activities for human diagnostic specimens. In some cases it may be advisable to perform initial processing of clinical samples in a biosafety cabinet, particularly if high levels of virus is suspected (such as tissues from fatal human cases). Biosafety Level 2 is recommended for processing field collected mosquito pools. Biosafety Level 3 and Animal Biosafety Level 3 practices, containment equipment, and facilities are recommended, respectively, for all manipulations of West Nile cultures and for experimental animal and vector studies. Containment specifications are available in the Centers for Disease Control and Prevention/National Institutes of Health publication Biosafety in Microbiological and Biomedical Laboratories (BMBL).[45] This document can be found online at both http://bmbl.od.nih.gov/ and http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

c) All bird necropsies should be done in a Class 2 biological safety cabinet.

2. Shipping of Agents

Shipping and transport of WNV and clinical specimens should follow current International Air Transport Association (IATA) and Department of Commerce recommendations. Because of the threat to the domestic animal population, a U.S. Department of Agriculture (USDA) shipping permit is required for transport of known WNV isolates. For more information, visit the IATA dangerous goods Web site at http://www.iata.org/cargo/dg/, and the USDA Animal and Plant Health Inspection Service (APHIS), National Center for Import /Export's Web site at http://www.aphis.usda.gov/ncie/.

B. Serologic Laboratory Diagnosis

Accurate interpretation of serologic findings requires knowledge of the specimen. For human specimens the following data must accompany specimens submitted for serology before testing can proceed or results can be properly interpreted and reported: 1) symptom onset date (when known); 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) state and county of residence; 5) travel history in flavivirus-endemic areas; 6) history of prior vaccination against flavivirus disease (e.g., yellow fever, Japanese encephalitis, or Central European encephalitis); and 7) brief clinical summary including clinical diagnosis (e.g., encephalitis, aseptic meningitis).

1. Human

a) Commercial kits for human serologic diagnosis of WNV infection are currently in development. Until these kits are available, the CDC-defined IgM and IgG ELISA should be the front-line tests for serum and CSF.[46-48] These ELISA tests are the most sensitive screening assays available. The HI and indirect immunofluorescent antibody (IFA) test may also be used to screen samples for flavivirus antibodies. Laboratories performing HI assays need be aware that the recombinant WNV antigens produced to date are not useful in the HI test; mouse brain source antigen (available from CDC) must be used in HI tests. The recombinant WNV antigen is available from commercial sources.

b) To date, the prototype WNV strains Eg101 or NY99 strains have performed equally well as antigens in diagnostic tests for WNV in North America.

c) To maintain Clinical Laboratory Improvements Amendments (CLIA) certification, CLIA recommendations for positive and negative ranges should be followed, and laboratories doing WNV testing should participate in a proficiency testing program through experienced reference laboratories; CDC's Division of Vector-Borne Infectious Diseases in Fort Collins, Colorado and the National Veterinary Services Laboratories in Ames, Iowa both offer this type of program.

d) Because the ELISA can cross-react between flaviviruses (e.g., SLE, dengue, yellow fever, WN), it should be viewed as a screening test only. Initial serologically positive samples should be confirmed by neutralization test. Specimens submitted for arboviral serology should also be tested against other arboviruses known to be active or be present in the given area (e.g., test against SLE, WN and EEE viruses in Florida).

2. Animal

a) In general, the procedures for animal serology should follow those used with humans cited above.

b) Plaque-reduction neutralization test (PRNT) and HI assays, although technically more demanding, may be useful because they are species independent.

C. Virologic Laboratory Diagnosis

Experience gained in WNV diagnostic testing over the past 4 years has led to the following recommendations:

1. Virus Isolation

a) Virus isolation attempts should be performed in known susceptible mammalian or mosquito cell lines. Mosquito origin cells may not show cytopathic effect and should be screened by immunofluorescence.

b) Appropriate samples for virus isolation are prioritized as follows:

1) Clinically ill humans - CSF (serum samples may be useful early in infection)

2) Human (biopsy or postmortem) - brain tissue

3) Horses (postmortem) - brain tissue (including brainstem), spinal cord tissue

4) Birds - kidney, brain, heart

5) Other mammals - multiple tissues, especially kidney and brain

c) Confirmation of virus isolate identity can by accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies, nucleic acid detection, or virus neutralization.

d) The IFA using well-defined murine monoclonal antibodies (MAbs) is the most efficient, economical, and rapid method to identify flaviviruses. MAbs are available that can differentiate WNV and SLE virus from each other and from other flaviviruses. Flavivirus-grouping MAbs are available for use as positive controls, and MAbs specific for other arboviruses can be used as negative controls. In addition, incorporating MAbs specific for other arboviruses known to circulate in various regions will increase the rapid diagnostic capacities of state and local laboratories. These reagents are available and should be used.

e) Nucleic acid detection methods including RT-PCR, TaqMan and nucleic acid sequence based amplification (NASBA) methods may be used to confirm virus isolates as WNV.

f) Virus neutralization assays also may be used to differentiate viruses, by using fourfold or greater titer differences as the diagnostic criterion in paired specimens (acute- and convalescent-phase).

2. Virus Detection in Tissues

a) <u>Antigenic analysis</u>

1) Immunohistochemistry (IHC) using virus-specific MAbs on brain tissue has been very useful in identifying both human and avian cases of WNV infection. In suspected fatal cases, IHC should be performed on formalin-fixed autopsy, biopsy, and necropsy material, ideally collected from multiple anatomic regions of the brain, including the brainstem, midbrain, and cortex.[24, 49]

2) Well-characterized antigen-capture ELISAs are now available for detection of SLE[50,51] and WNV antigen in mosquito pools and avian tissues.[25]

b) Nucleic acid analysis

A number of nucleic acid detection methods have recently been employed for WNV diagnostic and surveillance purposes. An independent antigen or nucleic acid test is required to confirm detection of WNV nucleic acid with any of these methods.

1) RT-PCR of tissues, mosquito pools, and CSF has proven to be a useful surveillance tool. RT-nested PCR has detected WNV nucleic acid in equine brain and spinal cord tissues. Standardized protocols developed by reference laboratories should be disseminated, and primer design information should be included so that other laboratories can prepare primers. A proficiency testing program should be developed by the reference laboratories so that these tests can be CLIA-certified in local laboratories.

2) Fluorogenic 5' nuclease techniques (real-time PCR) and nucleic acid sequence-based amplification (NASBA) methods have been developed and have undergone initial validation in specific diagnostic applications.[24,52-54]

D. *Training and Infrastructure*

1. State and Local Arbovirus Laboratories

Greater numbers of capable state and local laboratories performing screening assays (such as ELISA) should be developed to reduce time demands on reference laboratories. Reference laboratories should be utilized to confirm results of state and local laboratories, particularly for the initial identification of WNV in new locations and in new hosts.

2. Training Programs

Laboratory training programs have been developed and implemented at the federal level. Additional regional training programs may be beneficial.

III. PREVENTION AND CONTROL

Prevention and control of arboviral diseases is accomplished most effectively through a comprehensive, integrated mosquito management program using sound integrated pest management (IPM) principles.[55] IPM is based on an understanding of the underlying biology of the transmission system, and utilizes regular monitoring to determine if and when interventions are needed to keep pest numbers below levels at which intolerable levels of damage, annoyance, or disease occur. IPM-based systems employ a variety of physical, mechanical, cultural, biological and educational measures, singly or in appropriate combination, to attain the desired pest population control.

Programs consistent with best practices and community needs should be established at the local level and, at a minimum, should be capable of performing surveillance sensitive enough to detect West Nile Virus (WNV) enzootic/epizootic transmission that has been associated with increased risk of disease in humans or domestic animals. Integrated mosquito management programs designed to minimize risk of WNV transmission and prevent infections of humans and domestic animals should optimally include the following components (modified from information provided by the American Mosquito Control Association, the New Jersey Mosquito Control Association, and the Florida Coordinating Council on Mosquito Control)[56-58]

A. *Surveillance*

Effective mosquito control begins with a sustained, consistent surveillance program that targets pest and vector species, identifies and maps their immature habitats by season, and documents the need for control. Records should be kept on the species composition of mosquito populations prior to enacting control of any kind and to allow programs to determine the effectiveness of control operations. All components of the integrated management program must be monitored for efficacy using best practices and standard indices of effectiveness. The following is a list of surveillance methodologies used by mosquito control agencies.

1. Larval Mosquito Surveillance

Larval surveillance involves sampling a wide range of aquatic habitats for the presence of pest and vector species during their developmental stages. Most established programs have a team of trained inspectors to collect larval specimens on a regular basis from known larval habitats, and to perform systematic surveillance for new sources. A mosquito identification specialist normally identifies the larvae's species. Properly trained mosquito identification specialists can separate nuisance and vector mosquito species. Responsible control programs target vector and nuisance populations for control and avoid managing habitats that support benign species.

2. Adult Mosquito Surveillance

Adult mosquito surveillance is used to monitor species presence and relative abundance of adult mosquitoes in an area. Information derived from adult mosquito surveillance programs using standardized and consistent surveillance efforts provide information essential to monitoring potential vector activity, setting action thresholds, and evaluating control efforts. Various methods are available for this purpose and have been demonstrated to be effective in collecting a variety of mosquito species.[59] The New Jersey light trap, CDC's miniature light trap, and other modifications of this design, with or without carbon dioxide bait, have been used extensively for collecting host-seeking adult mosquitoes.[60] Gravid traps frequently are used to monitor the

ovipositing segment of *Cx. pipiens* and *Cx. restuans* populations. These species have been incriminated as the primary enzootic vectors of WNV in the northeastern states.[61,62] Host-seeking *Cx. tarsalis*, a species that has been strongly associated with WNV transmission in areas where this species is common, are readily collected in CO_2-baited CDC miniature light traps. Resting boxes frequently are used to measure populations of *Culiseta melanura*, a bird-feeding mosquito that is important in the amplification of eastern equine encephalitis (EEE) virus. Pigeon-baited traps may be employed to measure host-seeking *Culex* mosquitoes that amplify St. Louis encephalitis (SLE) and West Nile viruses. Day-active mosquitoes like *Ae. albopictus* are difficult to collect, and obtaining a sample representative of the local populations requires extra effort. Where these species are important, sample sizes may be enhanced by using CO_2-baited CDC miniature light traps during daylight hours or by using alternative trap configurations that may be more effective in collecting these species (e.g., Fay trap or traps using a counterflow geometry). Trap deployment should carefully address species habitat requirements on several spatial scales.

3. Virus Surveillance

The purpose of this component of the vector management program is to determine the prevalence of WNV in the mosquito population. This is often expressed simply as the number of WNV-positive mosquito pools of a given species collected at a defined location and time period. While the number of positive pools provides valuable information, it does not provide an index of virus prevalence in the vector population. Preferably, the proportion of the mosquito population carrying the virus should be expressed as the infection rate (IR, expressed as the estimated number of infected individual mosquitoes per 1,000 specimens tested). This is a more useful index of virus prevalence. The IR can be calculated by dividing the number of positive pools by the total number of specimens tested for that species and collection period, and multiplying the proportion by 1,000. This assumes that a positive pool contains only one infected mosquito, which is a valid assumption in most circumstances. When infection rates are high or sample sizes are low, a more accurate estimate of IR may be obtained by using a maximum likelihood estimate of the infection rate – see www.cdc.gov/ncidod/dvbid/westnile/software.htm. Elevated infection rates, particularly if sustained over several weeks or in populations of opportunistic blood-feeders that may act as bridge vectors, are indicators of increased WNV transmission risk. Specimens collected in the routine adult mosquito surveillance program plus special collections from key areas identified by other surveillance indicators (e.g., dead birds, sentinel flocks) can be used for this purpose. Mosquito collections made at permanent study sites in a sustained program provide important baseline data to which new surveillance data are compared and decisions about human risk and need for emergency interventions are made.

B. Source Reduction

Source reduction is the alteration or elimination of mosquito larval habitat breeding. This remains the most effective and economical method of providing long-term mosquito control in many habitats. Source reduction can include activities as simple as the proper disposal of used tires and the cleaning of rain gutters, bird baths and unused swimming pools by individual property owners, to extensive regional water management projects conducted by mosquito control agencies on state and/or federal lands. All of these activities eliminate or substantially reduce mosquito breeding habitats and the need for repeated applications of insecticides in the affected habitat. Source reduction activities can be separated into the following two general categories:

1. Sanitation

The by-products of human's activities have been a major contributor to the creation of mosquito breeding habitats. An item as small as a bottle cap or as large as the foundation of a demolished building can serve as a mosquito breeding area. Sanitation, such as tire removal, stream restoration, catch-basin cleaning and container removal, is a major part of all integrated vector management programs. Mosquito control agencies in many jurisdictions have statutory powers that allow for due process and summary abatement of mosquito-related public health nuisances created on both public and private property. The sanitation problems most often resolved by agency inspectors are problems of neglect, oversight, or lack of information on the part of property owners. Educational information about the importance of sanitation in the form of videos, slide shows**,** and fact sheets distributed at press briefings, fairs, schools and other public areas are effective.

2. Water Management

Water management for mosquito control is a form of source reduction that is conducted in fresh and saltwater breeding habitats. Water management programs for vector control generally take two forms, described below. Water management through impoundment and open water management have been very effective in the past. Recently, restrictions on modification of aquatic habitats have limited the implementation of these practices, and in many areas, water management for vector control is no longer routinely employed and may be impractical in many settings. In these situations, alternative methods of mosquito management must be employed.

a) <u>Impoundment Management</u>
Impoundments are mosquito-producing marshes around which dikes are constructed, thereby allowing water to stand or to be pumped onto the marsh surface from the adjacent estuary. This eliminates mosquito oviposition sites on the impounded marsh and effectively reduces their populations. Rotational Impoundment Management (RIM) is the technique developed to minimally flood the marsh during the summer months and then use flapgated culverts to reintegrate impoundments to the estuary for the remainder of the year, thereby allowing the marsh to provide many of its natural functions. Although impoundments usually achieve adequate control of salt-marsh mosquitoes, there are situations in which impoundments can collect stormwater or rainwater and create freshwater mosquito problems that must be addressed using other techniques.

b) <u>Open Marsh Water Management (OMWM)</u>
Ditching as a source-reduction mosquito control technique has been used for many years. Open marsh water management is a technique whereby mosquito-producing locations on the marsh surface are connected to deep-water habitat (e.g., tidal creeks, deep ditches) with shallow ditches. Mosquito broods are controlled without pesticide use by allowing larvivorous fish access to mosquito-producing depressions. Conversely, the draining of these locations occurs before adult mosquitoes can emerge. OMWM can also include establishing or improving a hydrological connection between the marsh and estuary, providing natural resource enhancement as well as mosquito control benefits. The use of shallow ditching (ditches approximately 3 feet or less in depth rather than the deep ditching used in

years past) is considered more environmentally acceptable because than deep ditching because fewer unnatural hydrological impacts occur to the marsh.

c) Management in Stormwater Retention Structures

Source reduction and water management practices may also be applied to stormwater retention structures designed to hold runoff before it is discharged into groundwater or surface water. Mosquito control should be considered in the design, construction, and maintenance of these structures, as appropriate. Stormwater retention structures should be designed in consultation with experts in mosquito biology and control to prevent as much mosquito production as possible, and to facilitate proper functioning and maintenance in the future. Regulations associated with stormwater retention and flood control structures should incorporate appropriate operations and maintenance provisions including considerations for routine monitoring and control of mosquito populations.

C. Chemical Control

Insecticides can be directed against either the immature or adult stage of the mosquito life cycle when source reduction and water management are not feasible or have failed because of unavoidable or unanticipated problems, or when surveillance indicates the presence of infected adult mosquitoes that pose a health risk. [63] Chemicals used by mosquito control agencies must comply with state and federal requirements. Public health pesticide applicators and operators in most states are required to be licensed or certified by the appropriate state agencies.

1. Larviciding

Larviciding, the application of chemicals to kill mosquito larvae or pupae by ground or aerial treatments, is typically more effective and target-specific than adulticiding, but less permanent than source reduction. An effective larviciding program is an important part of an integrated mosquito control operation. The objective of larviciding is to control the immature stages at the breeding habitat before adult populations have had a chance to disperse and to maintain populations at levels at which the risk of arbovirus transmission is minimal. Larvicides can be applied from the ground or by aerial application if large or inaccessible areas must be treated. Several materials in various formulations are labeled for mosquito larviciding including the organophosphate temephos (Abate); several biological larvicides such as *Bacillus thuringiensis israelensis* (*Bti*, a bacterial larvicide), *Bacillus sphaericus*; methoprene, an insect growth regulator (e.g., Altosid,); several larvicidal oils (e.g., petroleum-based Golden Bear and mineral-based Bonide) and monomolecular surface films (e.g., Agnique, Arosurf); and in some limited habitats diflubenzuron (e.g., Dimilin, a chitin synthesis inhibitor). Applications of larvicides often encompass fewer acres than adulticides because treatments are made to relatively small areas where larvae are concentrated, as opposed to larger regions where adults have dispersed. When applying larvicides, it is important that the material be specific for mosquitoes, minimize impacts on non-target organisms, and**,** where appropriate, be capable of penetrating dense vegetation canopies. Larvicide formulations (i.e., liquid, granular, solid) must be appropriate to the habitat being treated, accurately applied, and based on surveillance data. Accuracy of application is important because missing even a relatively small area can cause the emergence of a large mosquito brood resulting in the need for broad-scale adulticiding.

2. Adulticiding

Adulticiding is the application of pesticides to kill adult mosquitoes. The ability to control adult mosquitoes is an important component of any integrated mosquito management program, and like the other components of the program, its use should be based on surveillance data. Mosquito adulticiding may be the only practical control technique available in situations where surveillance data indicate that is necessary to reduce the density of adult mosquito populations quickly to lower the risk of WNV transmission to humans. In some situations, source reduction and larvicide applications are not practical, and adulticide application is the only available control strategy. Mosquito adulticides typically are applied as an Ultra-Low-Volume (ULV) spray where small amounts of insecticide are dispersed either by truck-mounted equipment or from fixed-wing or rotary aircraft.[64-68] Thermal fog applications of adulticides by ground or air are also used in some areas, but to a much lesser degree. Barrier treatments, typically applied as high volume liquids with hand-held spray equipment using compounds with residual characteristics, are common in some U.S. locations. This technique is especially attractive to individual homeowners living near mosquito-producing habitats where residual chemicals applied along property boundaries can provide some control benefits. Mosquito adulticiding differs fundamentally from techniques used to control many other adult insects. For adult mosquito control, insecticide must drift through the habitat in which mosquitoes are flying in order to provide optimal control benefits. The EPA has determined that the insecticides labeled nationally for this type of application do not pose unreasonable health risks to humans, wildlife, or the environment when used according to the label.[56] Adulticides labeled for mosquito control include several organophosphates such as malathion and naled. Some natural pyrethrins and synthetic pyrethroids (permethrin, resmethrin and sumithrin) also hold adulticide labels. Insecticide selection and timing of application should be based on the distribution and behavior of the target mosquito species. Application of adulticides should be timed to coincide with the activity period of the target mosquito species. Many *Culex* species are nocturnal and are active in the tree canopy level. This should be taken into consideration when planning adulticide applications. Operational experience indicates that *Cx. pipiens* and *Cx. quinquefasciatus* may require more frequent adulticide application to achieve desired levels of population reduction during an outbreak. Control of adult day-active species poses additional problems because ULV adulticide effectiveness is greatly reduced during daylight hours. Early-morning use of adulticides, applied before temperatures rise, may provide a measure of control for these species.

D. Resistance Management

In order to delay or prevent the development of insecticide resistance in vector populations, integrated vector management programs should include a resistance management component (modified from Florida Coordinating Council on Mosquito Control, 1998).[57] Ideally, this should include annual monitoring of the status of resistance in the target populations to:

1. Provide baseline data for program planning and pesticide selection before the start of control operations.

2. Detect resistance at an early stage so that timely management can be implemented (even detection of resistance at a late stage can be important in elucidating why disease control may fail); however, in such cases, management options other than replacement of the pesticide may not be possible).

3. Continuously monitor the effect of control strategies on resistance. In addition to monitoring resistance in the vector population, the integrated program should include

options for managing resistance that are appropriate for the local conditions.[69-70] The techniques regularly used include the following:

a) Management by Moderation - preventing onset of resistance by

1) Using dosages no lower than the lowest label rate to avoid genetic selection.

2) Using less frequent applications.

3) Using chemicals of short environmental persistence.

4) Avoiding slow-release formulations.

5) Avoiding the use of the same class of insecticide to control both adults and immature stages.

6) Applying locally**.** Currently, most districts treat only hot spots. Area-wide treatments are used only during public health alerts or outbreaks.

7) Leaving certain generations, population segments**,** or areas untreated.

8) Establishing high pest mosquito densities or action thresholds prior to insecticide application.

9) Alternation of biorational larvicides and insect growth regulators annually or at longer intervals.

b) Management by continued suppression - a strategy used in areas of high-value (e.g., heavily touristed areas) or where arthropod vectors of disease must be kept at very low densities.

This does not mean saturation of the environment by pesticides, but rather the saturation of the defense mechanisms of the insect by insecticide dosages that can overcome resistance. This is achieved by the application of dosages within label rates but sufficiently high to be lethal to susceptible as well as to heterozygous-resistant individuals. If the heterozygous individuals are killed, resistance (which is a homozygous trait) will be slow to emerge. This method should not be used if any significant portion of the population in question is resistant. Another approach more commonly used is the addition of synergists that inhibit existing detoxification enzymes and thus eliminate the competitive advantage of these individuals. Commonly, the synergist of choice in mosquito control is piperonyl butoxide (PBO).

c) Management by multiple attack - achieving control through the action of several different and independent pressures such that selection for any one of them would be below that required for the development of resistance.

This strategy involves the use of insecticides with different modes of action in mixtures or in rotations. There are economic problems (e.g., costs of switching chemicals or having storage space for them) associated with this approach, and critical variables in addition to mode of action must be taken into consideration (i.e., mode of resistance inheritance, frequency of mutations, population dynamics of the target species, availability of refuges, and migration). General recommendations are to evaluate resistance patterns at least annually and the need for rotating insecticides at annual or longer intervals.

E. Biological Control

Biological control is the use of biological organisms, or their by-products, to control pests. Biocontrol is popular in theory, because of its potential to be host-specific and virtually without non-target effects. Overall, larvivorous fish are the most extensively used biocontrol agent for mosquitoes. Predaceous fish, typically *Gambusia* or other species which occur naturally in many aquatic habitats, can be placed in permanent or semipermanent water bodies where mosquito larvae occur, providing some measure of control. Other biocontrol agents that have been tested for mosquito control, but that to date generally are not widely used, include the predaceous mosquito *Toxorhynchites*, predacious copepods, the parasitic nematode *Romanomermis,* and the fungus *Lagenidium giganteum*. Biocontrol certainly holds the possibility of becoming a more important tool and playing a larger role in mosquito control in the future, but will likely be effective only as part of an integrated approach.

F. Continuing Education of Mosquito Control Workers

Continuing education is directed toward operational workers to instill or refresh knowledge related to practical mosquito control. Training is primarily in safety, applied technology, and requirements for the regulated certification program mandated by most states.

G. Vector Management in Public Health Emergencies

A surveillance program adequate to monitor WNV activity levels associated with human risk must be in place. Detection of epizootic transmission of enzootic arboviruses typically precedes detection of human cases by several days to 2 weeks or longer (e.g., as found in SLE epidemics).[71,72] If adequate surveillance is in place, the lead time between detecting significant levels of epizootic transmission and occurrence of human cases can be increased, which will allow for more effective intervention practices.[19,27,31] Early-season detection of enzootic or epizootic WNV activity appears to be correlated with increased risk of human cases later in the season. Control activity should be intensified in response to evidence of virus transmission, as deemed necessary by the local health departments. Such programs should consist of public education emphasizing personal protection and residential source reduction; municipal larval control to prevent repopulation of the area with competent vectors; adult mosquito control to decrease the density of infected, adult mosquitoes in the area; and continued surveillance to monitor virus activity and efficacy of control measures.

As evidence of sustained or intensified virus transmission in an area increases, emergency response should be implemented. This is particularly important in areas where vector surveillance indicates that infection rates in *Culex* mosquitoes are increasing, or that potential accessory vectors (e.g., mammalophilic species) are infected with WNV. Delaying adulticide applications in such areas until human cases occur is illogical and negates the value and purpose of the surveillance system.

H. Adult Mosquito Control Recommendations

Ground-based (truck-mounted) application of adult mosquito control agents has several positive attributes. Where road access is adequate, such as in urban and suburban residential areas, good coverage may be achieved. In addition, ground-based application can be done throughout the night, thereby targeting night-active mosquito species. Such applications are prone to skips and patchy coverage in areas where road coverage is not adequate or in which the habitat contains significant barriers to spray dispersal and penetration.

Aerial application is capable of covering larger areas in shorter time periods than a ground-based application. This is a critical positive attribute when large residential areas must be treated quickly. In addition, aerial application is less prone to patchy coverage than ground-based application in areas where road coverage is not adequate. One limitation of aerial application is that many applicators will not fly at night, potentially reducing the effectiveness of the applications in *Culex* species control efforts. Cost benefits of aerial application over ground application may not be realized unless relatively large areas are treated.

Several formulations of a variety of active ingredients are available for adulticide applications. Material choice for ground-based or aerially applied mosquito control in public health emergency situations is limited by EPA restrictions on the pesticide label and applicable state and local regulations.

Multiple applications will likely be required to appreciably reduce *Culex* populations and interrupt arbovirus transmission. An emergency SLE virus response plan developed for New Orleans, Louisiana[63] indicates the need for repeated applications to control *Cx. quinquefasciatus*, and the need to repeatedly apply adulticides in high-risk areas (areas with human cases or positive surveillance events). Two to three adulticide applications spaced 3-4 days apart may be required to significantly reduce *Cx. pipiens* populations. Effective surveillance must be maintained to determine if and when re-treatment is required to maintain suppression of the vector populations.

Urban/suburban population centers with multiple positive surveillance events as described above should be treated first to most efficiently protect the largest number of people from exposure to WNV. Applications should be timed to coincide with the peak activity periods of the target species. For example, applications should be made at night to maximize control of night-active *Culex* species. Other species such as *Oc. sollicitans* or *Ae. vexans* are active shortly after sunset and are effectively controlled with appropriately timed applications. Day-active potential accessory vectors (e.g., *Oc. japonicus*, *Oc. triseriatus*, *Ae. albopictus*) must be addressed separately and are most effectively controlled by residential source reduction efforts, though there is preliminary evidence that early morning ULV applications may be used to control these species.

I. Determining the Scope of Mosquito Adulticiding Operations

Once arbovirus activity is detected in a jurisdiction and a decision is made to implement or intensify mosquito control by using adulticides, the size of the area to be treated must be determined. In the broadest context, the underlying program objective (i.e., interruption of the enzootic transmission cycle vs. prevention of transmission to humans and domestic animals) should determine the amount of adulticide coverage that is required. For most jurisdictions the objective is the prevention of transmission to humans and domestic animals. There is no simple formula for determining how large an area to treat around a positive surveillance indicator or a suspected or confirmed human case of WNV. Nor is there adequate information to guide decisions about the degree of vector population suppression that must be attained, or for how long this suppression must be maintained to reduce human disease risk. At a minimum, the following factors must be considered when deciding the scope of the adulticiding effort:

1. The general ecology of the area, e.g., key habitat types and the presence of natural barriers such as large rivers;

2. The population density, distribution, flight range, and age structure (proportion of parous females) of the target mosquito species;
3. The flight range of the avian amplifying host(s);
4. The length of time since birds started dying or became infected in the affected area (typically, there may be a lag of several weeks between recovery of dead birds and confirmation of WNV infection) or since virus-positive mosquito pools were collected;
5. The human population characteristics – spatial distribution and density relative to the positive locality (e.g., urban vs. rural), age demographics;
6. Evidence of persistent WNV activity detected by the surveillance program; and
7. Season of the year and how long WNV activity can be expected to persist until the epizootic/epidemic vector(s) enter diapause.

Several of these factors will be unknown or poorly understood. Technical assistance from a mosquito control professional, particularly one experienced in mosquito control in the region, is crucial in this process. Practical experience in conducting mosquito control is required to refine control recommendations. For example, the size of an area selected for control applications may be reduced in response to structures like open areas, bodies of water, major highways, or other barriers that may restrict the distribution of targeted species. Alternatively, adulticide coverage may be expanded to cover large urban or suburban residential neighborhoods with dense human populations.

Hypothetically, in some settings where focal early season enzootic WNV activity has been detected, early season adulticiding may be useful in interrupting virus transmission and lead to lower transmission rates later in the season. However, effective larval control of the principal enzootic mosquito vector is probably a more cost-effective way to interrupt early-season virus amplification.

J. Evaluation of Adult Mosquito Control

The following parameters should be periodically monitored during control operations:

1. Minimum requirements:

   a) Pre- and post spray vector mosquito densities inside and outside control area using CO_2-baited traps and gravid traps;
   b) Vector mosquito infection rates pre- and post-spray inside and outside the control area; and
   c) Weather conditions during application (temperature, wind speed, direction).

2. Desirable additions if capacity exists: population age structure of key mosquito species (*Cx. pipiens*)

3. In addition, both droplet size and flow rate should be documented for each piece of ULV application equipment:

4. During aerial application, GPS monitoring of spray track should be conducted if equipment is available on aircraft.

K. Health Education, Public Information, and Human Behavior Change

The goals of health education, public information, and behavior change programs are to

inform the public about WNV, promote the adoption of preventive behaviors that reduce disease risk, and gain public support for control measures. Health education/public information includes use of print materials (posters, brochures, fact sheets), electronic information (Web sites), presentations (health experts or peers speaking to community groups), and the media.

Information alone is seldom sufficient to encourage people to adopt new behaviors or to change old practices. Programs should include strategies to facilitate protective actions and to address barriers that hinder preventive actions. Examples of programs that go beyond information include developing a community task force, interventions to improve access to window screening materials or repellents, and social marketing to reinforce preventive behaviors.

The following section covers key prevention messages, selected best practices, and research/program development priorities for promotion of personal and community measures to decrease risk of WNV infection. Public education and risk communication activities must be ramped up to respond to the degree of WNV risk in a community, as noted in Table 1.

1. Key WNV Prevention Messages

a) Address the multiple levels at which prevention can occur: personal protection (use of repellent on skin and clothing, use of protective clothing, awareness of prime mosquito-biting hours); household protection (eliminating mosquito breeding sites, repairing/installing screens); and community protection (reporting dead birds, advocating for organized mosquito abatement, participating in community mobilization).

b) Use of DEET-based repellents on skin and clothing is the backbone of personal protection. (For current recommendations, see www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm.) Permethrin-based repellents should be promoted for use on clothing.

c) Emphasize the feasibility of actions that can lower an individual's WNV risk through personal protection measures. Messages should acknowledge the seriousness of the disease but should not be fear-driven. Fear-driven messages may heighten the powerlessness many people express in dealing with emerging diseases.

d) Recommendations to avoid being outdoors from dusk to dawn may conflict with neighborhood social patterns or practices of persons without air-conditioning or without other health programs seeking to increase physical activity. An alternative is to emphasize that the hours from dusk until dawn are prime mosquito-biting hours, and that protecting oneself through repellent use during these hours is important, with the option of remaining indoors.

e) Communication about adulticiding: Public acceptance of emergency adult mosquito control is critical to its success, especially where mosquito control is unfamiliar or unpopular. Questions about the products being used, their safety, and their effects on the environment are common. Improved communication about surveillance and how decisions to adulticide are made may help residents weigh the risks and benefits of control. When possible, provide detailed information regarding the schedule for adulticiding through newspapers, radio, the Internet, or a recorded phone message

f) Keep messages clear and consistent with the recommendations of coordinating agencies. Use plain language whenever possible, and adapt materials for lower-literacy and non-English speaking audiences.

2. Selected Best Practices

a) Targeted prevention

Audience members have different disease-related concerns and motivations for action. Proper message targeting permits better use of limited communication and prevention resources. The following are some audience groups that require specific targeting:

1) *Persons over age 50*: While persons of any age can be infected with WNV, US surveillance data indicate that persons over age 50 are at higher risk for severe disease and death due to WNV infection.

   Collaborate with organizations that have an established relationship with mature adults, such as the AARP, senior centers, or programs for adult learners. Include images of older adults in your promotional material. Identify activities in your area where older adults may be exposed to mosquito bites (e.g. jogging, golf, gardening).

2) *Persons with outdoor exposure:* While conclusive data are lacking, it is reasonable to infer that persons engaged in extensive outdoor work or recreational activities are at greater risk of being bitten by WNV-infected mosquitoes. Develop opportunities to inform people engaged in outdoor activities about WNV. Encourage use of repellent and protective clothing, particularly if outdoors during evening, night, or early morning hours. Local spokespersons (e.g., union officials, job-site supervisors, golf pros, gardening experts) may be useful collaborators.

3) *Homeless persons:* Extensive outdoor exposure and limited financial resources in this group present special challenges. Application of repellents with DEET or permethrin to clothing may be most appropriate for this population. Work with social service groups in your area to reach this population segment.

4) *Persons who live in residences lacking window screens:* The absence of intact window/door screens is a likely risk factor for exposure to mosquito bites. Focus attention on the need to repair screens and resources to do so. Partner with community organizations that can assist elderly persons or others with financial or physical barriers to screen installation or repair.

b) Partnerships with media and the community

Cultivate relationships with the media. Obtain media training for at least one member of your staff, and designate that individual as the organization's spokesperson. Develop clear press releases and an efficient system to answer press inquiries.

Develop partnerships with agencies/organizations that have relationships with populations at higher risk (such as persons over 50) or are otherwise recognized as community leaders (e.g., churches, service groups). Working through sources trusted by the target audience can heighten the credibility of and attention to messages. Partnerships with businesses that sell materials to fix or install window screens or that sell insect repellent may be useful in some settings.

c) Community mobilization and community outreach

Community mobilization can further education and behavior change goals. To

counter any idea that health departments/mosquito control programs are able to control WNV alone, develop community ownership for prevention activities. A community task force that includes civic, business, health, and environmental concerns can be valuable in achieving buy-in from various segments of society and in developing a common message. Community mobilization activities can include clean-up days to get rid of mosquito breeding sites.

Community outreach involves presenting messages in person, in addition to media and educational materials, and incorporating citizens in prevention activities. Hearing the message of personal prevention from community leaders can validate the importance of the disease. Health promotion events reinforce the importance of prevention in a community setting.

3. Research and Program Development Priorities

a) Audience research

Attitudes toward arboviral disease prevention vary considerably by region. Previous experience with nuisance mosquitoes and mosquito control will affect the acceptability of prevention efforts. Audience research can identify local attitudes, motivations, barriers to prevention, and opportunities to promote desired behaviors.

Audience research should ideally combine qualitative and quantitative efforts. Surveys assessing knowledge, attitude, and practice levels in the target population can be very helpful, especially in evaluation, though they are a substantial undertaking. Qualitative research techniques, such as interviews and focus groups, can yield valuable data, and are more adaptable to resource levels. Expertise to undertake such efforts may be available from other divisions within a health department (e.g., chronic disease programs, maternal and child health).

Pretesting of educational materials is an important step to ensure the usability of materials by the intended audience. Pretesting does not always have to involve considerable time or expense; simply having representatives of the intended audience review materials before printing will be useful.

b) Evaluation

Outcome evaluation should be conducted whenever possible to measure the efficacy of the intervention in achieving protective behaviors (e.g., frequency of repellent use, presence of household mosquito breeding sites). Outcome measurement requires extensive effort and must be planned from the outset of a program.

c) Social marketing and risk communication

The goal of social marketing is to achieve specific behaviors, using the concepts of product, price, place, and promotion. Use of social marketing approaches can help programs plan to achieve specific behavior change goals.

Risk communication is already used by many health departments, and can be useful in refining communication messages for WNV, especially as the disease becomes endemic in new areas, and in discussing community control. Risk communication can help people analyze the choices that are available to them and to their community.

4. Resources

The CDC Web site (www.cdc.gov/westnile) is updated frequently to reflect new findings and recommendations. Materials on the CDC Web site are generally in the public domain, and serve as a resource for state and local health departments and other organizations.

CDC staff can provide technical assistance in the development of audience research and strategies for public education and community outreach. Contact CDC/Division of Vector-Borne Infectious Diseases' health communication staff at 970-221-6400. CDC can provide other communication planning resources, including CDCynergy (www.cdc.gov/cdcynergy/), an interactive CD-ROM designed to help systematically plan health communication programs.

Other organizations that can provide useful information are the American Mosquito Control Association (www.mosquito.org/) and the National Pesticide Information Center (NPIC) (npic.orst.edu), a program of EPA and Oregon State University concerning pesticides and repellents. They can be contacted at 1-800-858-7378.

L. Legislation

In addition to statutes permitting legal action to abate mosquito-related public health nuisances, legislation must be in place to allow creation of and provide funding for municipally-based integrated mosquito management programs. Local jurisdictions can contact state mosquito control associations to provide examples of enabling legislation.

M. Guidelines for a Phased Response to WNV Surveillance Data

The principal goal is to minimize the health impact of the WNV in humans, as well as in domestic and zoo animals. Given the limited understanding of the ecology and epidemiology of WNV in the U.S., the low incidence of arboviral encephalitis, and the limitations of prevention methods, prevention and control measures, regardless of intensity, may not prevent all WNV infections in humans.

The recommended response levels for the prevention and control of WNV should augment, but not replace, long-standing mosquito control efforts by established programs. These programs often have two objectives: 1) to control nuisance mosquitoes, and 2) to control vector mosquitoes that can transmit pathogenics. Nuisance mosquito control often has different objectives than vector control, and the target mosquito species may also differ. Established mosquito control programs often have long-standing experience with the surveillance and control of indigenous neurotropic arboviruses such as SLE virus. These programs have established thresholds for response based on historical data. Long-standing experience with WNV does not exist in the U.S.

These guidelines for the prevention and control of WNV should be interpreted according to the following considerations:

1. All states should prepare for WNV activity. Given the extensive geographic spread of WNV since 1999, its occurrence in many different habitats and ecosystems in the Old World, its expansion into numerous habitat types in the Western Hemisphere, and the fact that SLE virus, a related flavivirus, is widespread in the U.S., there appear to be no barriers to the spread of WNV throughout the U. S. At a minimum, a plan for the

surveillance, prevention, and control of WNV should be developed at the state and local levels.

2. Measures of the intensity of WNV epizootic in an area should be considered when determining the level of the public health response. Accumulating data analyses indicate that intensity of epizootic WNV activity as measured by avian mortality and mosquito infection rates are good indicators of subsequently increased human infection risk. Data from NYC indicate that human WNV disease cases were more likely to occur in counties that had experienced more than 0.1 dead crow reports per square mile per week. In the Staten Island outbreak of 2000, the density exceeded 1.5 dead crow reports per square mile per week. Also, analysis of 2001 and 2002 surveillance data indicate that counties reporting WNV-infected dead birds early in the transmission season are more likely to report subsequent WNV disease cases in humans than are counties that do not report early WNV-infected dead birds. These observations should be interpreted as a guide rather than an absolute. Levels of epizootic activity that correlate with increased human risk will vary by region.

3. Flexibility is required when implementing the guidelines. Knowledge gained from ongoing surveillance and research could change the phased response recommendations. Specific and detailed recommendations that will fit all possible scenarios are not possible, particularly at a local level. Therefore, public health action should depend on interpreting the best available surveillance data in an area, in light of these general guidelines. In addition, the following factors should be considered when translating these guidelines into a plan of action:

a) Current weather and predicted climate anomalies;

b) Quality, availability, and timeliness of surveillance data;

c) Feasibility of the planned prevention and control activities, given existing budgets and infrastructure;

d) Public acceptance of the planned prevention and control strategies;

e) Expected future duration of WNV transmission (surveillance events earlier in the transmission season will generally have greater significance); and

f) Other ongoing mosquito control activities, such as nuisance mosquito control or vector mosquito control for the established arboviral encephalitis viruses.

The recommended phased response to WNV surveillance data is shown in Table 1. Local and regional characteristics may alter the risk level at which specific actions must be taken.

Table 1. Suggested Guidelines for Phased Response to WNV Surveillance Data

Risk category	Probability of human outbreak	Definition	Recommended response*
0	None	Off-season; adult vectors inactive; climate unsuitable.	Develop WNV response plan. Secure surveillance and control resources necessary to enable emergency response. Initiate community outreach and public education programs. Conduct audience research to develop/ target education & community involvement. Contact community partners.
1	Remote	Spring, summer, or fall; areas anticipating WNV epizootic based on previous WNV activity in the region; no current surveillance findings indicating WNV epizootic activity in the area.	Response as in category 0, plus: conduct entomologic survey (inventory and map mosquito populations, monitor larval and adult mosquito density), Initiate source reduction; use larvicides at specific sources identified by entomologic survey and targeted at likely amplifying and bridge vector species; Maintain avian mortality, vector and virus surveillance; Expand community outreach and public education programs focused on risk potential and personal protection, and emphasizing residential source reduction; Maintain surveillance (avian mortality, mosquito density /IR, human encephalitis/meningitis and equine illness).
2	Low	Summer, or fall; areas with limited or sporadic WNV epizootic activity in birds and/or mosquitoes. No positives prior to August.	Response as in category 1, plus: increase larval control, source reduction, and public education emphasizing personal protection measures, particularly among the elderly. Enhance human surveillance and activities to further quantify epizootic activity (e.g., mosquito trapping and testing). Implement adulticide applications if vector populations exceed locally established threshold levels, emphasizing areas where surveillance indicates potential for human risk to increase.
3	Moderate	Spring, summer, or fall; areas with initial confirmation of epizootic WNV in birds before August; a horse and/or a human case, or sustained WNV activity in birds and/or mosquitoes.	Response as in category 2, plus: intensify adult mosquito control in areas where surveillance indicates human risk, Initiate adult mosquito control if not already in progress, Initiate visible activities in community to increase attention to WNV transmission risk (speaker, social marketing efforts, community mobilization for source reduction, etc.), Work with collaborators to reduce risks to elderly (e.g., screen repair).
4	High	Spring, summer, or fall; quantitative measures indicating WNV epizootic activity at a level suggesting high risk of human infection (*e.g.*, high dead bird densities In early summer, sustained high mosquito infection rates, multiple positive mosquito species, horse or mammal cases indicating escalating epizootic transmission, or a human case and high levels of epizootic activity). Areas with early season positive	Response as in category 3, plus: Expand public information program to include TV, radio, and newspapers (use of repellents, personal protection, continued source reduction, risk communication about adult mosquito control), Increase visibility of public messages, engage key local partners (e.g., government officials, religious leaders) to speak about WNV ; intensify and expand active surveillance for human cases; intensify adult mosquito control program, repeating applications in areas of high risk or human cases.

		surveillance indicators where WN epidemic activity has occurred in the past.	
5	Outbreak in progress	Multiple confirmed cases in humans; Conditions favoring continued transmission to humans (*e.g.*, persistent high infection rate in mosquitoes, continued avian mortality due to WNV)	Response as in category 4, plus: Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. Enhance risk communication about adult mosquito control. Monitor efficacy of spraying on target mosquito populations. If outbreak is widespread and covers multiple jurisdictions, consider a coordinated widespread aerial adulticide application; emphasize urgency of personal protection through community leaders and media, and emphasize use of repellent at visible public events.

- Local and regional characteristics may alter the risk level at which specific actions must be taken.

IV. HEALTH DEPARTMENT INFRASTRUCTURE

State and Local Health Departments

In the 48 contiguous United States, state and local health departments should have a functional arbovirus surveillance and response unit, staffed by well-trained personnel who have adequate data-processing resources, appropriate laboratory facilities, and an adequate operating budget. The size and complexity of these units will vary by jurisdiction, depending on both the risk of arboviral transmission in the area and available resources. A functional arbovirus surveillance unit at the state level should be considered an essential component of any emerging infectious diseases program. Local health department expertise and capabilities should be supported in a manner that complements statewide programmatic goals.

A. Staffing and Personnel

Ideally, arboviral surveillance involves epidemiologists, virologists, medical entomologists, vertebrate biologists, veterinarians, laboratory staff, environmental toxicologists, public affairs personnel, and data managers. In a particular jurisdiction, the combination of personnel needed to conduct arboviral surveillance will depend on the importance of arboviral diseases in the area and on resources. Many health departments experience a chronic shortage or complete absence of medical entomologists and expertise in wildlife pathobiology. Addressing these deficiencies should be a high priority. In the event of an arboviral disease outbreak, local health departments will likely require significant surge capacity to ensure an adequate public health response. Contingency planning to identify resources to assist with the enhanced surveillance, laboratory, environmental, and public health needs should be identified ahead of time.

B. Training and Consultation

Opportunities exist at federal and state agencies for appropriate training of and consultation with laboratorians, medical entomologists, epidemiologists, vertebrate biologists, and others involved in arbovirus surveillance.

C. Laboratory Capacity

The infrastructure of arbovirus laboratories in the U.S. has deteriorated significantly in recent decades, not only in terms of the total number of functional laboratories and overall capacity, but also in terms of the staffing, physical plant, and financial support of many remaining laboratories. This is a problem of national scope and significance, the solution for which will require leadership at all levels of government.

1. Testing for West Nile Virus (WNV) Infections

In the wake of the introduction of WNV into the Western Hemisphere, it is important to distinguish between increasing short-term and long-term laboratory capacity. The latter is preferred and should be emphasized over the former. Laboratories with an existing capability for arbovirus serology should consider adding serologic screening tests for WNV to their repertoire. For serologic screening of patients and mosquito pools, arrangements can be made with CDC to transfer existing technology and reagents, and to obtain appropriate training. Samples giving positive or equivocal screening results should be confirmed by CDC or another laboratory capable of definitive testing. For selected laboratories, similar technology transfer arrangements can be made with regard to RT-PCR primers for use in the testing of tissues and mosquito pools. In the wake of the recent epidemic of WN encephalitis in the Northeast, it is important that

programs continue to routinely test for other arboviruses historically active in their area, such as St. Louis encephalitis, eastern equine encephalitis, western encephalitis, and La Crosse viruses, as well as for other causes of acute encephalitis.

D. Developing Local Public Health Agency Infrastructure

The function of local public health agencies is assessment, assurance, and policy development to promote and protect the health of the public. As part of this function, local public health agencies are responsible for preventive activities to reduce the risk of WNV infection to individuals in their jurisdictions. This responsibility includes educating communities about reducing mosquito breeding sites and taking personal protective measures. Local public health agencies also must have the capacity to assess human risk by gathering surveillance data or having access to surveillance data gathered on a district, regional, or statewide basis. These local public health agencies are important to formulating local recommendations on the indications and decisions concerning mosquito adulticiding. Education of and communication with the public, and maintenance of local media contacts are generally primary functions of the local public health agency. Included in this responsibility is communicating risk regarding the use of pesticides.

The following infrastructure and functional capacities fall within the province of local public health agencies. Where these are not directly provided, access to these capacities is to be ensured).

1. Risk assessment based on surveillance data (including mosquito, bird, and human data). Surveillance data may also include reports from individuals or healthcare providers indicating possible adverse health effects from pesticide use.

2. Health education regarding personal protection, reduction of mosquito breeding sites and minimum health risks posed by approved pesticides applied according to the label.[73,74]

3. Communication with the media.

4. Development of a preventive plan including education, mosquito source reduction, and larviciding.

5. Public response capability, particularly when surges of public inquiries arise. This may include the use of telephone hotlines and Internet Web sites.

6. Training of staff.

7. Coordination with state and federal agencies.

8. Local coordination by formulation of a task force with organizations such as departments of public works, offices of public affairs, city/county building management, departments of parks and recreation, departments of planning and zoning, property or building inspection services, police, public schools, colleges and universities, nonprofit and grassroots organizations, businesses, zoos, animal/vector control, local mosquito control districts, emergency medical services, hospitals, poison control centers, departments of game and inland fisheries, departments of environmental quality, emergency, management agencies, etc.

V. INTERJURISDICTIONAL DATA SHARING AND NATIONAL REPORTING OF HUMAN CASES

The public and animal health response to West Nile virus (WNV) epidemics/epizootics involves all levels of government, including the federal governments of the U.S. and neighboring countries, and the Pan American Health Organization. In addition, multiple government agencies at each level are often involved. Rapid, efficient, secure, and coordinated systems are needed to allow the sharing of human and ecologic data between these multiple agencies to support long-term surveillance activities, and to support activities that are part of the rapid outbreak response.

During an epidemic involving multiple jurisdictions, CDC staff and other authorized persons will use Epi-X, a CDC-sponsored, Web-based system for secured electronic communication, or similar integrated communication systems, for rapid dissemination of information on public health events of public health significance.

A. Human Epidemiological, Clinical, and Laboratory Data Collection

Patient confidentiality statutes vary among jurisdictions. Data can be shared between jurisdictions if recipients agree to adhere to the confidentiality statutes of the jurisdiction providing the data. Electronic databases should be appropriately secured by passwords to limit access and minimize opportunities for breaches in confidentiality or security.

B. National Reporting of Human WNV Disease Cases

1. National Reporting of Human Cases of West Nile Meningoencephalitis (WNME)

WNME is included in the list of nationally notifiable diseases maintained by the Council of State and Territorial Epidemiologists (CSTE) in consultation with CDC. CDC has designated 10056 as a specific disease code ("EVENT" code) for use in reporting WNME cases via the National Electronic Telecommunications System for Surveillance (NETSS). For national reporting purposes, states should use the national surveillance case definition of arboviral encephalitis/meningitis for classifying cases as either confirmed or probable (see Appendix C). Until such time as ArboNET and NETSS are consolidated under the National Electronic Disease Surveillance System (NEDSS) standards, duplicate reporting of human cases of WNME to both ArboNET and NETSS will be encouraged.

2. West Nile Fever (WNF)

Although WNF is not included in the list of nationally notifiable diseases, states are encouraged to report WNF cases to CDC via ArboNET, using a CDC recommended case definition (see Appendix D). States may also choose to report WNF cases to NETSS using EVENT code 10049.

C. Ecologic Data

Many of the issues that apply to the interjurisdictional sharing of human data apply to the sharing of ecologic data as well, although key differences exist. For example, confidentiality is generally not an issue with nonhuman cases, particularly wild animals identified as part of a surveillance program. Maintaining confidentiality may be important for certain owned animals. Data standardization is a far more challenging issue because of the relatively large number of species being studied. Specific needs include the following:

1. **Accurate Taxonomic Identification of Specimens**

   Fully understanding the epidemiology and developing effective prevention and control strategies for WNV requires accurate identification of all animal species involved in the virus transmission and maintenance cycles. This is especially true for birds and mosquitoes.

2. **Unique Identifier (UID) Numbering System for Specimens**

   A UID numbering system should be used in each jurisdiction (e.g., state, county, city, surveillance area). Such a system should distinguish readily between each major animal group reported (i.e., humans, birds, and mosquitoes), and encode the location of collection (county or town), date of collection (day/month/year), and a specimen-specific number.

3. **Durable Tagging System for Field-Collected Specimens**

   Use appropriate labels containing complete specimen information on all samples (blood, tissues, or whole animals) so field specimen identification will not be lost during shipment to testing facilities.

VI. RESEARCH PRIORITIES

The human and animal health implications of the introduction of West Nile virus (WNV) to the U.S. and to the Western Hemisphere continue to emerge. Many questions remain, the answers to which will require considerable research. A research agenda should be supported, with priority given to research questions whose answers can be directly applied to prevention and control.

A. Current and Future Geographic Distribution of WNV

To determine the geographic distribution of WNV in the Western Hemisphere, existing laboratory-based surveillance systems for WNV in human, birds, other selected animals, and mosquitoes should be enhanced, or new, active systems should be developed and implemented (see Section I).

B. Bird Migration as a Mechanism of WNV Dispersal

Experience in Europe and the Middle East suggests that WNV regularly is introduced to new geographic areas along bird migration routes. A better understanding of this potential is required for the Western Hemisphere. Studies should include the frequency and duration of chronic infections that will allow the long-range transport and recrudescence of viremias necessary to infect mosquitoes.

C. Vector and Vertebrate Host Relationships and Range

Relatively little is known about the vertebrate host and mosquito vector relationships of WNV in the U.S. and the Western Hemisphere. Effective prevention and control strategies will require targeting selected species involved in maintenance, epidemic/ epizootic transmission cycles, or both. It is critical that the principal species and the range of these species be determined.

D. Virus Persistence Mechanisms

It is not known whether or how WNV will be maintained in the U.S. over the long term. Overwintering mechanisms in *Culex* and *Aedes* species should be investigated, as well as persistence and maintenance of the virus in ticks. Other possibilities that should be investigated include the duration of chronic infection and reactivation in birds or other animals, and the introduction of the virus by migratory birds.

E. Mosquito Biology, Behavior, Vector Competence, Surveillance, and Control

It is critical that a better understanding is gained of the principal mosquito vectors involved in maintenance, bridge (from enzootic to peridomestic), and epidemic/epizootic transmission. Different vector species may be important in different geographic or ecologic regions. Understanding their biology and behavior will allow for more effective surveillance and development of targeted control methods.

F. Development and Evaluation of Prevention Strategies

Effective prevention and control of WNV transmission will require evaluation of the efficacy of current control methods and research on new and innovative control strategies for the principal mosquito vectors. Ultimately, prevention strategies must be integrated and use a variety of approaches to control mosquitoes and reduce the risk of transmission. Research should also be conducted to better define target areas for mosquito control in response to documented WNV activity in an area.

A very long-term goal is the identification and implementation of new, natural compounds to repel and control mosquito vectors of disease. With efforts to decertify current pesticides, new compounds will be needed in the fight against vector-borne diseases.

Much effort has been expended to increase public awareness of the WNV threat and of the actions needed to reduce exposure to infected mosquitoes. These actions include using mosquito repellents, reducing periresidential mosquito breeding sites, and wearing protective clothing when entering mosquito-infested areas. The success of these public information campaigns has not been formally evaluated using scientific instruments such as knowledge and behavior surveys. The cost of such campaigns is high, so formal attempts to assess their success are needed.

G. Laboratory Diagnosis

Surveillance for WNV will continue to require accurate laboratory diagnostic tests. Ideally, these tests will be simple and inexpensive, and will distinguish between WNV and other flaviviruses such as the SLE, dengue, and yellow fever viruses. Virus-specific tests for IgM or IgG antibody will be required for humans, various species of birds, horses, and other mammals. Sensitive viral detection methods will be required for both human and animal tissues as well as for mosquito pools.

H. Clinical Spectrum of Disease and Long-Term Prognosis in Humans

A better understanding of the spectrum of illness caused by WNV infection in humans is needed, including the long-term consequences of acute infection of the central nervous system. In addition to the severe end of the clinical spectrum (viral encephalitis), it is important to know the degree to which mild viral syndromes occur and whether these patients have any unique clinical presentations that may be characteristic or even pathognomonic. It is also important to know whether they have viremia and, if so, its magnitude and duration. Effective clinical management of severe disease will require detailed clinical studies of confirmed human cases of WNV infection.

I. Risk Factor Studies

Data on the risk factors associated with human and animal infection with WNV are required to develop more effective prevention strategies, particularly when educating the public to take specific prevention measures to reduce exposure to infection.

J. Detailed Clinical Descriptions and Outcome in Human Cases

Larger and more detailed case series, as well as studies of short- and long-term outcomes, are needed to better understand the clinical features, clinical course, and public health impact of WNV disease in humans. A suggested framework for collecting standardized “extended” clinical variables is included in Appendix E.

K. Viral Pathogenesis

Little is known of the pathogenesis of WNV in humans or other animals. Research is needed to better understand the organ systems affected, the mechanism of central nervous system (CNS) infection, and the role of virus strain in pathogenesis.

L. Genetic Relationships and Molecular Basis of Virulence

Only since 1996 has WNV been associated with significant numbers of severe disease cases and fatalities in humans. It is important to better understand whether genetic changes in WN viruses influence their phenotypic expression (i.e., host and vector range, clinical expression in various hosts, and epidemic potential). This will require detailed studies of the genome of WN virus strains isolated from different epidemics in various geographic areas.

M. Vaccine Development for Animals and Humans

Ultimately, the most effective prevention strategy may be vaccination. It is important to support research on the development of both human and equine vaccines.

N. Antiviral Therapy for West Nile Virus and Other Flaviviruses

To date, none of the available antiviral agents are effective against flaviviruses, including WNV. Research in this area is critical to effective management of severe disease in humans.

O. The Economic Cost of the WNV Epidemic/Epizootic

It is important to estimate the total economic cost of the epidemic/epizootic. These data will help set priorities for capacity building and prevention programs.

P. WNV Impact on Wildlife

WNV has the potential to greatly impact the wildlife populations in the Western Hemisphere. This is especially true for birds, in many of which the infection appears to have high mortality rates (i.e., Corvidae). Research is needed to analyze and define this impact to determine if the development of new epizootic intervention strategies is needed. Research is also needed to determine what long-term effects WNV infection may have on its animal hosts.

Q. Investigate Alternate Modes of WNV Transmission to Humans

Four new modes of WNV transmission to humans were identified in 2002: blood transfusion, tissue transplantation, transplacental transfer, and breast-feeding. New modes of transmission should be investigated to determine the impact they have on human infection and to develop effective approaches for prevention and control of WNV infection by these routes.

Appendix A - National WNV Surveillance System

Objectives:

The objectives of the national West Nile virus (WNV) surveillance system are to:

- Monitor the geographic and temporal spread of WNV in the U.S.
- Develop national public health strategies for WNV surveillance, prevention, and control.
- Develop a more complete regional picture of the geographic distribution and incidence of the other clinically important arboviruses in the U.S.
- Provide national and regional information to public health officials, elected government officials, and the public.
- Evaluate the use of cooperative agreement funds and the need for additional resources.

Scope:

Coordinated, multi-state surveillance of WNV infections in humans and animals has been repeatedly identified as a high priority by states affected by WNV in 1999-2002. All states conducting surveillance for WNV and other arboviruses are encouraged to participate in ArboNET, a CDC-coordinated program to collect these surveillance data. While the components of WNV surveillance systems employed in individual jurisdictions will vary, national WNV surveillance should, at a minimum, focus on collection of data from:

- Mosquito surveillance
- Avian (dead bird) surveillance
- Equine surveillance
- Human surveillance

In addition to data from states, data from commercial laboratories will be sought. CDC will 1) formally notify all such laboratories of the need to report any positive laboratory results to the appropriate state or local health department who, in turn, will notify CDC; 2) provide them with a list of state health department contact persons; 3) periodically contact them to encourage reporting; and 4) remind them of the need to have all positive screening tests for arboviral infections confirmed by state public health laboratories. In addition, CDC will provide a list of these commercial laboratories to its cooperative agreement partners, to facilitate their efforts to conduct active laboratory-based surveillance for arboviral infections.

Categories of Data to be Collected:

National surveillance will focus on the collection of two general categories of data:

- *"Denominator"* data

  Definition: Weekly totals of dead birds (classified as either corvids or 'others') and mosquito pools (classified by species) collected and/or tested by a jurisdiction's WNV surveillance system, stratified by county within a state. Because recent experience has demonstrated that the following categories of denominator data are of limited use in meeting national surveillance goals, as of 2003, CDC will discontinue the collection of totals of sentinel and free-ranging wild birds, horses, or other non-human mammals tested.

- *"Numerator"* data

  Definition: Detailed information on individual mosquito pools, sentinel species, dead birds, and ill humans, horses, or other species with confirmed or suspected WNV infections, as determined by laboratory-confirmed or -probable test results.

General Procedures:

Reporting "denominator" data:

CDC will collect aggregate denominator data via a secure file upload system using a state-based database provided by CDC, continuous data entry into a database stored on a secured CDC web site, or importation of delimited records in a specified format. Denominator data variables are specified in Table 1. An appropriate submission schedule will be arranged by CDC with the jurisdictions submitting surveillance data via file uploading. In addition,

- CDC will distribute the necessary software and provide the adequate licenses that will allow regular secured file upload or continuous web-based data entry.

- CDC will accommodate state health departments with existing integrated data collection systems, *e.g.*, by arranging for uploads of XML-formatted data.

- The data entry screens will be designed as a series of simple forms or tables.

- The system will accommodate updates and corrections of previously transmitted data by jurisdictions.

- Following the entry of a week's data into the database at the state level, transmission of the data file to CDC will involve a minimal number of keystrokes. Security will be insured by use of the sender's digital certificate. CDC will arrange for those who will be transmitting surveillance data to CDC to obtain digital certificates.

- Upon arrival at CDC, records from the specific reporting week of interest will automatically be captured and imported into a master database on the CDC fileserver and also transmitted to USGS in Reston, Virginia.

- Using these data, reports will be generated automatically each week. Maps will be generated by CDC and USGS and made available on the USGS web site. A basic set of dynamic maps and corresponding graphs and tables will be made available weekly. The CDC web site and Epi-X (or a similar secured communication network) will contain links to the relevant USGS web pages.

Reporting "numerator" data:

CDC strongly encourages prompt ("real-time") reporting of numerator data. CDC will collect such reports in a standardized manner to allow monitoring of regional and national trends, and facilitate prompt confirmatory testing when necessary. As the arbovirus transmission season progresses, the need for immediate reporting of certain data to CDC may diminish. For example, once numerous WNV-positive mosquito pools have been previously documented in a given geographic area, there may not be a compelling need to *immediately* report further findings. In addition, if at any time the volume of reporting becomes overwhelming, adoption of an alternative system may be necessary.

Numerator data variables that will be collected are specified in Table 2. WNV laboratory and surveillance case criteria are specified in Table 3.

Specified, line-listed numerator data may be submitted using one of three methods:

- Web-based data entry to a CDC server;
- Use of state-based, CDC-distributed, Microsoft Access-based data entry/management software (ArboNET) with continuous file upload to a CDC server; or
- Data messaging from a unique data collection system to a CDC server (*e.g.,* in XML format).

All data entry will be done by the reporting jurisdiction and data is transmitted to a CDC server. After data entry and submission, numerator data will be available on the CDC Secure Data Network (SDN) so that authorized personnel from the reporting jurisdiction may "verify" (proofread, correct, and clear for publication) individual numerator data records in selected surveillance categories.

It is essential that each numerator data record include a unique identifier (UID) assigned by the reporting state agency. UIDs will be used by CDC staff to track and update individual numerator data records, and by states to verify records via the CDC SDN. The UID will not appear in output products for public release. Most jurisdictions already have systems in place for generating UIDs, and they should continue to use them. CDC's databases will accommodate numeric or alphanumeric UIDs up to 25 characters long. Jurisdictions are encouraged to begin their UIDs with their state's 2-letter postal code (or "NYC" for New York City).

The issue of numerator data records associated with laboratory-probable results deserves special mention. Although CDC encourages confirmation of all laboratory-probable results, it is realized that under some circumstances some states may choose not to do so, depending on the epidemiologic situation, laboratory capacity, and volume. For example, during a known WN viral epizootic, a state may decide that a crow brain associated with a single positive result for WN viral RNA by RT-PCR will undergo no further testing. Although this bird is a laboratory-probable case (see table below), the jurisdiction may decide to upload that bird's numerator data record to CDC and subsequently authorize CDC to release it publicly. In contrast, a jurisdiction may opt to delay the release of such results to the public until they have been laboratory-confirmed. CDC will rely on individual jurisdictions to decide when to authorize the public release of numerator data records based on *laboratory-probable* results.

CDC will not publicize numerator data records associated with laboratory-equivocal results.

In terms of human surveillance, the national surveillance case definition of arboviral encephalitis/meningitis includes two official case-status categories: confirmed and probable (Table 3). For national arboviral encephalitis surveillance, CDC has traditionally combined records in

these two categories for its annual summary reports, and will continue this practice within the WNV surveillance system. States are encouraged to promptly report both laboratory-confirmed and laboratory-probable human WN encephalitis cases as numerator data records.

CDC encourages the reporting of human WN viral illnesses other than WNME (*e.g.*, WNF, acute flaccid paralysis, other clinical syndrome, or unspecified). To determine case status (confirmed or probable) for reporting purposes, refer to the national surveillance case definition of arboviral encephalitis/meningitis (Appendix C) and the CDC-recommended surveillance case definition for WNF (Appendix D). A working case definition for WNME in equines is shown in Appendix B.

Arboviruses other than WNV:

It is anticipated that enhanced WNV surveillance will result in increased recognition of other domestic arboviral activity, including eastern equine encephalitis (EEE), western equine encephalitis (WEE), SLE, La Crosse (LAC), and Powassan (POW) virus activity. Surveillance numerator (laboratory-positive) data regarding these viruses may be reported to CDC/DVBID via ArboNET, telephone, FAX, or e-mail.

Data Security Issues:

General principles:

- State and local health authorities will retain control of the timing of data release.

- As of 2003, reporting agencies will electronically report to ArboNET all categories of surveillance data, including human numerator data. For non-human data, agencies will verify accuracy and readiness for public release prior to submission. Upon the electronic submission of non-human data to CDC, these reports will be considered verified and publishable. With the 2003 version of ArboNET, human data will be automatically verified upon entry, and the reporting agency has the option to unverify the data via electronic checkbox. CDC will not publicly release unverified human case reports.

- Personal identifying or localizing (more specific than county) information will not be released.

Specific issues:

- To report data via secure file upload to the CDC fileserver or to enter data directly onto a secured web site, states will utilize the CDC SDN, which provides data encryption for transmission via the Internet. To use the SDN, users must obtain and install a digital certificate from the CDC certificate server. This allows for unique identification of the computer/browser that is accessing a secure web site.

- To obtain a digital certificate and be approved to use the SDN, the digital certificate authority at CDC/DVBID must approve the request and forward it to CDC/Atlanta. CDC requests that <u>a maximum of 3</u> persons from each state be designated to receive digital certification. These should include those who will transmit data to CDC, as well as those who will verify data on the SDN.

Summary Reports to be Produced by CDC and USGS:

A working list of basic summary reports is shown in Table 4. The exact list and formats of these

reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may take place over time, as dictated by feedback, experience, technical issues, and events.

Using state-approved numerator and denominator data, reports will be generated weekly. Maps and tables will be generated by DVBID and by USGS. Maps and corresponding graphs and tables will be updated at least weekly on the USGS web site (www.USGS.gov).

Communication Issues:

- A dedicated telephone line (970-266-3592), electronic mailbox (dvbid2@cdc.gov), and fax machine (970-266-3599) will be available at CDC/DVBID (in Fort Collins, Colorado) 24 hours/day for reporting numerator data or other urgent WNV-related business. During nights and weekends, calls to the dedicated phone line will be forwarded to the cellular phone of an on-call CDC/DVBID staff scientist. *Because of potential delays in the receipt and reading of email and fax messages, in general please use the telephone for time-sensitive business.*

- In addition to periodic conference calls between CDC, cooperating states, and other federal agencies, Epi-X and the WNV Information Exchange (WNVIX, part of the Epi-X Forum) will be available to participating jurisdictions and agencies using the CDC SDN. For further information, contact the CDC/DVBID ArboNET staff at 970.221.6400 or send electronic mail to dvbid2@cdc.gov.

Submission of Laboratory Specimens to CDC for WNV Testing:

See Table 5.

Table 1. *Denominator* Data Variable List

(Note: As of 2003, *denominator* data will no longer be collected in the following categories: sentinel animals, seroprevalence in free-ranging birds, and ill equines or humans.)

I. *Avian mortality:* (Includes ill or dead birds, except for sentinels.)

? Year

? MMWR week that bird collected ("MMWR week collected")
(Note: "MMWR week collected" corresponds to the earliest date associated with a specimen. Preferably, this should be MMWR week that corresponds *to the date that the bird was reported by the public*. But, if a date of report is not available, use the MMWR week that corresponds to the *date that the specimen was collected in the field*. This "MMWR week collected" should remain associated with this specimen **throughout testing.**)

? County

? State

? Number of reported corvids by "MMWR week collected" and by county (Data source: State, county or township WNV surveillance coordinators through the state to CDC)

? Number of corvids **tested** by "MMWR week collected" and by county (Data source: Testing laboratories through state)

? Number of other reported birds by "MMWR week collected" and by county (Data source: Jurisdictional WNV surveillance coordinators to CDC via state or municipal health departments)

? Number of other birds **tested** by "MMWR week collected" and by county (Data source: Testing laboratories through state)

Note: Laboratory-positive" results are reported through the *numerator* system by the testing facility/agency. In this report, the date of reporting/sighting or field collection is routinely obtained. By definition, each *numerator* data record of a WNV-positive dead bird should also be included within an aggregated *denominator* data record.)

II. *Mosquito collections:*

? Year

? MMWR week of collection
(Note: This is the MMWR week that corresponds to the date of field collection. This date should remain associated with this specimen **throughout testing.**)

? County

? State

? Species of mosquito

? Number of mosquitoes collected by MMWR week of collection, by county, and by species (Data source: Jurisdictional WNV surveillance coordinators to CDC via state or municipal health departments)

? Number of mosquitoes **tested** by MMWR week of collection, by county, and by species (Data source: Testing laboratories through state).

(Note**:** Laboratory-positive results are reported through the *numerator* system by the testing facility/agency. In this report, the date of field collection is routinely obtained. By definition, each *numerator* data record of a WNV-positive mosquito pool should also be included within an aggregated *denominator* data record.)

Table 2. *Numerator* data variables

Mosquito surveillance – state, county, pool UID, date of mosquito collection, week of collection, species, arbovirus, case status

Sentinel species surveillance - State, county, group UID, date of serum collection, week of serum collection, species, arbovirus, case status

Avian mortality surveillance – state, county, bird UID, week bird found collected, date bird collected, species (including "captive species"), arbovirus, case status

Avian seroprevalence surveillance – state, county, bird UID, week bird trapped & bled, date bird trapped & bled, species, arbovirus, case status

Veterinary (non-avian) surveillance – state, county, animal UID, week of illness onset, date of illness onset, species (canine, equine, feline, bat, squirrel, rabbit, raccoon, or other species), arbovirus, case status.

Human surveillance – state, county, patient UID, week of illness onset, date of illness onset, imported from, arbovirus, case status, age, age unit, birthdate, sex, race, ethnicity, clinical syndrome, fatality, date of death, lab acquired, non-lab acquired, blood donor, blood recipient. organ donor, organ transplant recipient, breast fed infant at time of illness, potential in-utero infection, pregnant at time of illness

Table 3. WNV Laboratory and Surveillance Case Criteria

Laboratory case definitions:

Surveillance Type	Laboratory-confirmed WNV infection	Laboratory-probable WNV infection*
Mosquito	• WNV isolation (identity of virus established by at least two of the following techniques: • Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive test using different primers, 2) positive PCR result using another system (*e.g.*, TaqMan), or 3) virus isolation. • Detection of WN viral antigen (*e.g.,* IFA, EIA, VecTest™) validated by inhibition test (for ELISA), RT-PCR, or virus isolation	• Positive RT-PCR test for WN viral RNA in a single test • Antigen detection not validated by another procedure
Sentinel species	• WNV isolation, RNA detection, or antigen detection as described for mosquitoes, • Seroconversion to WNV in serially collected serum specimens, by plaque-reduction neutralization** • Detection of IgM antibody to WNV, validated by demonstration of neutralizing antibody to WNV**	• Detection of IgM antibody to WNV • Seroconversion to WNV in serially collected serum specimens, strongly reactive by EIA or IFA
Avian mortality	• WNV isolation, RNA detection, or antigen detection as described for mosquitoes, ,	• Positive RT-PCR test for WN viral RNA in a single test • Antigen detection not validated by another procedure

Surveillance Type	Laboratory-confirmed WNV infection	Laboratory-probable WNV infection*
Veterinary (non-avian)	• As for humans (see below)	• As for humans (see below)
Human	• See national surveillance case definitions (Appendices C and D)	• See national surveillance case definitions (Appendices C and D)

* CDC strongly encourages attempts to confirm all laboratory-probable and -equivocal results. Further testing of laboratory-probable human specimens will depend on availability of confirmatory testing.

** SLE virus infection should be ruled-out by cross-neutralization; criterion for PRNT positive is a 90% neutralization titer of at least 1:10, and 4-fold greater titer compared to other flaviviruses such as SLE.

Table 4. Working List of Basic Weekly Summary Reports to be Produced by CDC

NOTE: The exact list and formats of these reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may occur over time, as dictated by feedback, experience, technical issues, and events.

A. National map: U.S. map with state boundaries reflecting cumulative data.
   1. Mosquito surveillance:
      a. Map showing each state's counties as WNV-positive, WNV-negative, or blank (no data)
   2. Sentinel chicken surveillance:
      a. Map showing each state's counties as WNV-positive or blank (no data)
   3. Avian morbidity/mortality surveillance:
      a. Map showing each state's counties as WNV-positive, WNV-negative, or blank (no data)
   4. Veterinary (non-avian) surveillance:
      a. Map showing each state's counties as WNV-positive (# cases) or blank (no data)

   5. Human surveillance:
      a. Map showing each state's counties as WNV-positive (# cases) or blank (no data)

B. State Maps: Selecting an individual state from the national map will produce a map of that state with its county boundaries indicating the positive specimens reported for that county and an accompanying table of cumulative positive specimens reported by county.
   1. Mosquito surveillance:
      a. Map showing each county as WNV-positive with a count of positive specimens reported, WNV-negative, or blank (no data)
   2. Sentinel species surveillance:
      a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data) by sentinel species (*e.g.*, horse, chicken)
   3. Avian mortality surveillance:
      a. Map showing each county as WNV-positive with a count of positive specimens reported, WNV-negative, or blank (no data)
   4. Veterinary (non-avian) surveillance:
      a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data)
   5. Human surveillance:
      a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data)

Table 5. Instructions for Submitting Laboratory Specimens to CDC for WNV Testing

Arrangements for Testing:

Mosquito specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector surveillance coordinator. For specimens considered by a state health department vector surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Roger Nasci, tel. 970-221-6432, RNasci@cdc.gov; if Dr. Nasci cannot be reached, please phone 970-266-3592.

Sentinel chicken specimens: Serum specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector or vertebrate surveillance coordinator. For specimens considered by a state health department vector or vertebrate surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

Avian morbidity/mortality specimens: On a case-by-case basis, special arrangements can by made for CDC to conduct initial and/or confirmatory tests of tissue specimens (especially brain, heart, kidney, and spleen) from dead birds that cannot otherwise be tested in state health department laboratories or by the National Wildlife Health Center, USGS. For further information, please contact Dr. Nick Komar, tel. 970-221-6496, NKomar@cdc.gov; if Dr. Komar cannot be reached, please call 970-266-3592.

Veterinary (non-avian) specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For routine testing of veterinary specimens, contact the state health department laboratory or the National Veterinary Services Laboratory, USDA, in Ames, IA (Tel. 515-663-7751), or another collaborating laboratory. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of that state's public health laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

Human specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

General Shipping Instructions:

All shippers should adhere to International Air Transport Association regulations (*http://www.iata.org*).

Specimens should be shipped by overnight courier to arrive at CDC on Tuesday-Friday. *Always notify CDC staff in advance of an impending shipment* (tel. 970-221-6445; if no answer, phone 970-266-3592). Do not ship specimens on Friday unless special arrangements have been

made.

Shipping address: CDC/DVBID
CSU Foothills Campus/Rampart Road
Fort Collins, CO 80521
ATTENTION: Arbovirus Diagnostic Laboratory (tel. 970-221-6445)

Shipping containers: Use only durable containers. Seal specimen containers tightly. Wrap specimen containers in absorbent material and pack them into two different plastic containers to insure that any leakage is contained. Specimens for virus isolation must be sent on enough dry ice to insure that they remain frozen until receipt. Specimens for serologic testing can be shipped on gel-ice and need not remain frozen. Hand-carrying specimens is not recommended but if specimens are hand-carried, the above packing instructions are applicable.

Minimal Information to Accompany Specimens Shipped to CDC:

See information in columns 2, 3, and 4 in Table 2. Please read carefully and supply all available information. Use CDC Form 5034 (the ADASH@ form) Form 5034 is available electronically at: *http://www.cdc.gov/ncidod/dvbid/CDC_form5034.pdf*

Tubes, cryovials, and other specimen containers should be clearly labeled with – at minimum – the specimen's UID, patient's name (human), state, date of onset, date of collection, and specimen type.

Special Collection, Shipping, and Handling Instructions:

Mosquitoes: Ship on dry ice.

Serum: Store in externally threaded plastic tubes. Ship at least 0.5 mL per specimen. Whenever possible, acute and convalescent specimens should be shipped together. Ship fresh-frozen on dry ice (required for virus isolation) or refrigerated on wet ice (acceptable).

CSF: Store in externally threaded plastic tubes. Ship at least 1.0 mL per specimen. Ship fresh-frozen on dry ice (required for virus isolation) or refrigerated on wet ice (acceptable).

Whole blood: In general, send only if requested for virus isolation attempts in fatal cases (heart blood).

Pregnancy-related specimens: In possible cases of intrauterine arboviral infection, tissues collected at the time of delivery can be tested for evidence of infection. The following tissues should be shipped fresh-frozen on dry ice: cross-sections of umbilical cord, placental tissue (approximately 1 cm^3 per sample), cord serum and maternal serum (0.5 ml each), and colostrum or breast milk. For more information, please contact Dr. Dan O'Leary at (970) 266-3525 or DOLeary@cdc.gov.

Autopsy specimens: In suspected cases of arboviral encephalitis in which an autopsy is performed, **fresh-frozen** tissues can be tested, including brain (multiple areas of cortex, midbrain, brainstem, and spinal cord), other solid organs (liver, spleen, pancreas, heart, kidney, etc.), CSF (collected from ventricles), and heart blood (for virus isolation attempts).

After consulting with Dr. Sherif Zaki or other CDC/Atlanta pathology staff member**s** (tel. 404-639-3133), **tissue samples suspended in formalin** should be sent to:

Infectious Disease Pathology Activity
DVRD/NCID/CDC
Building 1, Room 2301
1600 Clifton Road, N. E.
Atlanta, GA 30333

Veterinary (non-avian) tissues: As for human specimens.

Avian tissues: Submit fresh-frozen brain, heart, kidney, and spleen samples.

Appendix B B Surveillance Case Definition for WNV Infection in Equines

Laboratory criteria for diagnosis

Compatible clinical signs[1] plus one or more of the following:

- Isolation of West Nile (WN) virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF) or other body fluid;[2] or
- Detection of IgM antibody against WN virus by IgM-capture ELISA in serum (at 1:400 or greater dilution) or cerebrospinal fluid (CSF) (at dilution 1:2 or greater dilution); or
- An associated 4-fold or greater change in IgG-capture ELISA or plaque-reduction neutralization test (PRNT) antibody titer to WN virus in appropriately timed,[3] paired serum specimens from an equid that is unvaccinated against WN virus; or
- Positive immunohistochemistry (IHC) for WN virus antigen in tissue.

Case classification

Probable: compatible clinical signs occurring during a period when arboviral transmission is likely, and with the following supportive serology: 1) a single or stable (less than or equal to two-fold change) but elevated titer of WN virus-specific IgM-capture ELISA or neutralizing serum antibodies without knowledge of prior WN virus vaccination.

Confirmed: compatible clinical signs with laboratory-confirmed evidence of WN virus infection.

Notes:

[1] Clinical signs are associated with central and/or peripheral nervous system dysfunction. Most horses exhibit secondary CNS-derived neurological manifestations such as ataxia (including stumbling, staggering, wobbly gait, or incoordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, altered mental status, blindness, lip droop/paralysis, teeth grinding. (Ostlund et al, Equine West Nile Encephalitis, United States, Emerging Infectious Diseases, Vol 7, No 4. Jul – Aug 2001) Fever is not a consistent finding.

[2] Preferred diagnostic tissues from equids are brain or spinal cord; isolation of WN virus or detection of WN viral nucleic acid sequences in equine blood or CSF are infrequent. (Bunning et al, Experimental Infection of Horses with *West Nile virus,* Vol 8, No. 4. April 2002)

[3] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least 14 days post-onset.

Assumptions on which case definitions are based:

- IgM-capture ELISA testing may give nonspecific results; cross-reactions to closely related flaviviruses (e.g., St. Louis encephalitis virus) may occur. Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (*e.g.*, in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conduction cross-neutralization tests using an appropriate battery of closely related viruses.

- Vaccination refers to one or more doses of the current USDA-licensed inactivated WN virus vaccine.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means infection with WN virus or a closely related flavivirus has occurred, probably within the last three months. (personal communication Eileen N. Ostlund, USDA**)**
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WN virus; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.
- Neutralizing antibody detected in serum by PRNT indicates past infection with WN virus or vaccination with WN virus vaccine; equines exposed to WN virus in prior years may test positive by PRNT.

Appendix C - National Surveillance Case Definition for Arboviral Encephalitis/Meningitis, 2001

(available at http://www.cdc.gov/epo/dphsi/casedef/encephalitiscurrent.htm)

Encephalitis or Meningitis, Arboviral (includes California serogroup, Eastern equine, St. Louis, Western equine, West Nile, Powassan)

2001 Case Definition

Clinical description

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis. Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g., paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements).

Laboratory criteria for diagnosis

- Fourfold or greater change in virus-specific serum antibody titer, or
- Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or
- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
- Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition).

Case classification

Probable: an encephalitis or meningitis case occurring during a period when arboviral transmission is likely, and with the following supportive serology: 1) a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or 2) serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.

Confirmed: an encephalitis or meningitis case that is laboratory confirmed.

Comment

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with WN (or dengue) virus, or vice versa, in areas where both of these viruses occur.

The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Reporting should be etiology-specific (see below; the six encephalitides/meningitides printed in bold are nationally reportable to CDC):

St. Louis encephalitis/meningitis (NETSS Event Code: 10051)

West Nile encephalitis/meningitis (NETSS Event Code: 10056)

Powassan encephalitis/meningitis (NETSS Event Code: 10057)

Eastern equine encephalitis/meningitis (NETSS Event Code: 10053)

Western equine encephalitis/meningitis (NETSS Event Code: 10052)

California serogroup viral encephalitis/meningitis (includes infections with the following viruses: La Crosse, Jamestown Canyon, snowshoe hare, trivittatus, Keystone, and California encephalitis viruses) (NETSS Event Code: 10054)

Other viral CNS infections transmitted by mosquitoes, ticks, or midges (*e.g.*, Venezuelan equine encephalitis/meningitis [NETSS Event Code: 10055] and Cache Valley encephalitis/meningitis [NETSS Event Code: 10058])

Appendix D - CDC-Recommended Surveillance Case Definition for WN Fever

What is a CDC-Recommended Case Definition?
CDC-recommended surveillance case definitions are prepared for use by U.S. States and Territories interested in conducting public health surveillance for diseases or conditions that have not been designated nationally notifiable and have not been officially approved and sanctioned by the Council of State and Territorial Epidemiologists (CSTE). A CDC-recommended case definition may not be approved by CSTE in the future, unless CSTE and the CDC program with responsibility for prevention and control of the selected disease or condition both wish to seek broader and more formalized approval from both organizations.

CASE DEFINITION

Case Description

A non-specific, self-limited, febrile illness caused by infection with WNV, a mosquito-borne flavivirus. Clinical disease generally occurs 2-6 days (range, 2-15 days) following the bite of an infected mosquito. Typical cases are characterized by the acute onset of fever, headache, arthralgias, myalgias, and fatigue. Maculopapular rash and lymphadenopathy generally are observed in less than 20% of cases. Illness typically lasts 2-7 days.

Case Classification

A clinically compatible illness, *plus*:

Confirmed:

1) Fourfold or greater change in WNV-specific serum antibody titer;

2) Isolation of WNV from or demonstration of specific WN viral antigen or genomic sequences in tissue, blood, CSF, or other bodily fluid; or

3) WNV-specific IgM antibodies demonstrated in serum by antibody-capture enzyme immunoassay and confirmed by demonstration of WNV-specific serum neutralizing antibodies in the same or a later specimen.

Probable:

1) WNV-specific serum IgM antibodies detected by antibody-capture enzyme immunoassay but with no available results of a confirmatory test for WNV-specific serum neutralizing antibodies in the same or a later specimen.

(Note: Some WN fever cases progress to WN meningitis or encephalitis. Cases meeting the more restrictive case definition of WN encephalitis/meningitis should be reported as such and only once, using event code 10056 for "WN Encephalitis or Meningitis".)

Comment

The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (*e.g.*, in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to identify the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against WNV are not the result of an infection with St. Louis encephalitis or dengue virus, or vice versa. Because dengue fever and WN fever can be clinically indistinguishable, the importance of a recent travel history and appropriate serologic testing cannot be overemphasized. In some persons, WNV-specific serum IgM antibody can wane slowly and be detectable for more than one year following infection. Therefore, in areas where WNV has circulated in the recent past, the co-existence of WNV-specific IgM antibody and illness in a given case may be coincidental and unrelated. In those areas, the testing of serially collected serum specimens assumes added importance.

Date case definition was developed: October 2002
Event Code: 10049
Source of the case definition: National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases, Arbovirus Diseases Branch.
Questions and comments about the case definition should be directed to the following CDC/ADB staff:

Roy Campbell	Phone: (970) 221-6459	E-mail: glc5@cdc.gov
Dan O'Leary	Phone: (970) 266-3525	E-mail: dbo7@cdc.gov
Tony Marfin	Phone: (970) 266-3521	E-mail: aam0@cdc.gov

Appendix E – Recommended Framework for Standardized "Extended" Clinical Variables in Studies of Human WNV Disease

Larger and more detailed case series, as well as studies of short- and long-term outcome, are needed to better understand the clinical features, clinical course, and public health impact of WNV disease in humans. A suggested framework for collecting standardized "extended" clinical variables is shown below. During 2003, CDC will work with its partners to populate this framework with specific questions in each category. The use of standardized questions will allow public health officials and other researchers to compare results more readily.

1. Epi core data (e.g., age, gender, residence location, race/ethnicity, type of West Nile virus illness, etc.) *[Note: These are already standard ArboNET variables.]*
2. Past medical history
3. Previous arboviral infections or vaccinations
4. Immunosuppressed conditions
5. New modes of transmission
6. Clinical presentation – neurology and initial symptoms
7. Clinical presentation – Standardized scale of neuro/physiologic function (e.g., APACHE, Glasgow Coma Scale, PRISM)
8. Clinical presentation – laboratory
9. Clinical presentation – WNV diagnostic studies
10. Clinical presentation – Special diagnostic studies (e.g., MRI, EEG, EMG, lumbar puncture)
11. Treatment (e.g., antivirals, steroids, anti-seizure medications, hyperventilation, interferon, intravenous immunoglobulin, plasmapharesis)
12. Clinical course (e.g., renal function, electrolyte balance, neurologic complications)
13. Morbidity (e.g., number of hospital-, ICU-, and ventilator-days, number and type of nosocomial infections, etc.) *[Note: Many of the morbidity parameters can be used in determining the costs of WNV disease.]*
14. Nosocomial infections
15. Discharge disposition
16. Neurologic and functional status at disposition, at 90 days post-discharge, and 180 days post-discharge
17. Mortality (e.g., cause of death, pathology findings, etc.)

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INTERNATIONAL PERSPECTIVES

Although most of the information presented in this journal refers to situations within the United States, environmental health and protection issues are boundaryless. The journal periodically runs International Perspectives to ensure that readers are aware of conditions, experiences, and lessons learned that may increase or lessen risks of harm to all our readers, no matter where they live or work.

Eco-Friendly Control of Mosquito Larvae by *Brachytron pratense* Nymph

S.N. Chatterjee, M.Sc., Ph.D
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Abstract

The study reported here revealed the biocontrol efficacy of aquatic nymphs of the dragonfly *Brachytron pratense* against larvae of the mosquito *Anopheles subpictus*. It was found that during a 24-hour study period, a nymph of *B. pratense* would consume (mean value of three observations) 96 fourth-instar *An. subpictus* larvae released in a water bowl containing 3 liters of pond water. The consumption rate was significantly higher ($p < .05$) during the lights-on phase of the experiment than during the lights-off phase ($t = 2.13$). Under field conditions, a significant decrease ($p < .05$) in larval density in dipper samples was observed 15 days after the introduction of dragonfly nymphs (10 individuals) in concrete tanks. The biocontrol potential of the nymphs under field conditions was also indicated by a significant increase ($p < .05$) in the density of mosquito larvae 15 days after the removal of nymphs. In the control tanks (where no nymphs were introduced), mean larval-mosquito density did not differ significantly throughout the study period ($p > .05$).

Introduction

Although dragonflies are often associated with ponds, streams, and swamps, they have rarely been studied as a potential biocontrol agent against malaria and other insectborne diseases. Dragonflies belong to the order Odonata, which includes dragonflies (suborder Anisoptera) and damselflies (suborder Zygoptera). Worldwide, nearly 5,000 species of dragonfly are known (Bevier & Wiens, 1970). The principal methods of distinguishing the various dragonfly families are based on wing venation, eye structure, color, and size. *B. pratense* belongs to the Darner family (Aeshnidae) of dragonflies and is commonly known as the "hairy hawker dragonfly" because of the numerous long hairs on its thorax.

B. pratense feeds voraciously on insects during its larval stages as well as in its adult stage. Its larger size allows for greater consumption. The natural feeding behavior of the nymph is also innately effective. The nymphs of various dragonfly species generally feed using one of three techniques, passively feeding from burrows in the bottom sediment (burrowers), hunting from the bottom sediment surface (sprawlers), or actively stalking their prey among the vegetation. The nymphs of *B. pratense* have good vision and are vegetation stalkers that actively hunt prey.

The life cycle of dragonflies is relatively long, and the nymph stage, consisting of 10 to 15 nymphal instars between egg and imago, may last for one year or more (Tillyard, 1917). The presence of dragonflies is not seasonal, but the adults are found in greater numbers during summer and monsoon seasons (March through September). Dragonflies have some preference with respect to habitat (preferring mesotrophic habitats), but the factors responsible for this preference are not clearly understood. The presence or absence of wild vegetations in and around ponds and lakes may be one factor affecting the level of nutrients in the water.

Anopheles subpictus is a house-frequenting mosquito among the anophelines reported from various parts of the world. It has also been reported as a malaria vector in the Austral-Asian zone (Russel, West, Manwell, & Macdonald, 1963) and as a secondary vector of malaria in Sri Lanka. It has also been reported as a malarial vector in various parts of India (Kulkarni, 1993; Pandey, Geetha Bai, Bhima Rao, Viswam, & Suryanarayanamurthy, 1981; Roy, 1943). In the Jadavpur area of West Bengal, India, this mosquito species is very prevalent and acts as a primary vector of malaria (Chatterjee & Chandra, 2000). It is resistant to chemical insecticides in Pakistan, Nepal, Sri Lanka, Indonesia, and Bangladesh, as well as in various parts of India such as Delhi state, Punjab, and Pondicherry (Das, Chandrahas, & Panicker, 1980; Rahman, Taqin, & Roisen, 1985; Sharma, 1996). *Anopheles subpictus* breeds mainly in the small ponds and submerged fields of the study area. Although the larvae are present throughout the year, more than 70 percent of them occur during the rainy season (July to October) (Chatterjee and Chandra, 2000).

Although most of the information presented in the journal refers to situations within the United States, environmental health and protection issues are boundless. The journal periodically uses international perspectives to create dialogue on environmental conditions, approaches and risk assessment methods, and challenges readers to make direct comparisons of issues and results, irrespective of origin.

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Mosquitoes serve as a vector not only for malaria, but also for yellow fever, dengue, filariasis, encephalitis, and other diseases. Owing to growing resistance to commonly used organochlorine and organophosphate chemicals in the field, control of mosquitoes through eco-friendly biological means may be advantageous. Other benefits of biocontrol agents include an ability to kill the targeted species while being safe for nontargeted ones, ease of field application, inexpensive production, and the lack of infectivity and pathogenicity to mammals, including humans (Rabbow et al., 1988; World Health Organization [WHO], 1975a; WHO, 1989). The alternative malaria control strategy of bio-environmental improvement techniques gives primary importance to antilarval operations.

Although the larvae of *Toxorhynchites* sp. (mosquito) and *Diplonychus indicus* (water bug) have been considered as potential biocontrol agents against mosquitoes (Sankaraganesh & Venkatesan, 1989; Steffan & Evenhuis, 1981), information on the predatory instinct of dragonfly nymphs with respect to mosquito larvae is rare (Fincke, Yanoviak, & Hanschu, 1997; Hati & Ghosh, 1995; Pritchard, 1964). The purpose of the study reported here was to observe the daily feeding rate of the *Brachytron pratense* nymph on fourth-instar *An. subpictus* larvae. The efficacy of these nymphs in controlling mosquito larvae under field conditions also was observed.

Methods

Laboratory-Based Experiment

An. subpictus larvae, which had been obtained from various sources of clean unpolluted water in Burdwan, West Bengal, were colonized and maintained from 2000 continuously for several generations in a laboratory free of exposure to pathogens, insecticides, or repellents. The laboratory colony was maintained at a temperature of 25–30°C, relative humidity of 85–90 percent, pH of 6.95–7.03, and dissolved oxygen of 5.5–6.1 mg/l, in the insectary of the Mosquito Research Unit, Department of Zoology, University of Burdwan. Under these conditions, full development from egg to adult lasted about two to three weeks. Larvae were fed on finely ground dog biscuit. The adult colony was provided with 10 percent sucrose and 10 percent multivitamin syrup, and was periodically blood-fed on restrained rats. Fourth-instar larvae were continuously available for the experiments. The fourth-instar larval form was selected because it is the last prepupal instar, with the highest survival rate among all the larval instars and the lowest likelihood of being consumed by a predator because of its maximum size.

The nymphs of *Brachytron pratense* were also collected from shallow freshwater ponds and rice fields in Burdwan and were placed separately in 5-L glass jars and maintained with a diet consisting of midge larvae (family Chironomidae) and dried yeast powder for three days. In the laboratory, one *B. pratense* nymph (4.5 cm in length, as measured by a stereoscope, Olympus microscope [10x]) was allowed to feed on 100 fourth-instar *An. subpictus* larvae for 24 hours in a 5-L water bowl containing 3 L of pond water. The pond water, which was similar in quality to that of the insects' habitat, was sieved through a net (300 mesh) to exclude larvae of other insects. The temperature of the water ranged from 21 to 23°C, the relative humidity ranged from 59 to 66 percent, the pH from 6.63 to 8.87, and dissolved oxygen from 5.23 to 8.19 mg/l, during the experiment. The numbers of *An. subpictus* larvae consumed by nymphs of each dragonfly species during the lights-on phase (09:00–17:00, Indian Standard Time [IST]) and the lights-off phase (17:00–09:00, IST) were reported at 3-hour intervals for 24 hours. The authors poured the water through a fine-mesh sieve to collect all mosquito larvae, which were transferred to a white pan for counting. After each 3-hour interval, the glass jar was replenished with the number of larvae that had been consumed, along with the same volume of water, to maintain the same prey density. The lengths of the lights-on and lights-off phases were maintained by the application of artificial lights (white fluorescent lights) set on the walls of the laboratory (six 40-watt bulbs). The lights-on phase in the laboratory was manually synchronized with the natural outdoor sunlight photophase and the lights-off phase with the dark phase of the night. The experiment was conducted three times on three separate days (n = 3) with the same nymph to generate an arithmetic mean.

Field-Based Experiment

To examine the efficacy of dragonfly nymphs in field conditions, the authors selected the communities of Kalna and Burdwan in the District of Burdwan, West Bengal, India, as study areas. In both areas, 10 outdoor open concrete tanks were selected, each with the capacity to hold approximately 300 L of water. These tanks are usually used in the processing of rice (paddy). They remain unused for a long time each year—that is, from after the rainy season to early spring—and play the role of a natural breeding place for local mosquitoes (mainly comprising *An. vagus*, *An. subpictus*, *An. barbirostris*, *An. annularis*, *Armigeres subalbatus*, *Culex quinquefasciatus*, *Cx. vishnui*, *Cx. tritaeniorhynchus*, and *Cx. vishnui*).

Each water tank was manually sieved to ensure that it was free from any larvae or nymphs of larvivorous insects or leaves. This step used fine netting (with >130 mesh) that allowed only the passage of mosquito larvae. After filtering, the debris was collected and washed away to keep the standard quality of the water. Before the field experiments were conducted, the larval density in each of the tanks was assessed with a 250-mL dipper (WHO, 1975b). Each time, 30 dips (n = 30) were taken, and the mean prevailing larval density was calculated. Ten healthy collected nymphs of *B. pratense* were introduced into each of five tanks (tank numbers 1 to 5). No nymphs were released into the remaining five tanks (tank numbers 6 to 10), which were kept as controls. Larval densities in all the tanks were assessed 15 days after the introduction of dragonfly nymphs and on the day nymphs were removed. Larval densities in all the tanks were assessed again 15 days after the removal of nymphs. Data were analyzed statistically by the application of a two-tailed t-test.

The experiments both in laboratory and in the field were conducted in the months of August and September 2004. During the field experiments, the temperature of the water was 19–23°C, the pH 6.23–6.79, the relative humidity 55–63 percent, and dissolved oxygen 5.16–6.23 mg/l.

Results

B. pratense nymphs are aquatic; during the laboratory-based experiments, they crawled at the bottom of the water bowl and came to the surface to capture *An. subpictus* larvae with their modified labium, which was drawn out into a prehensile organ called the mask. While they were catching the prey, this mask was thrown forward, and with incredible swiftness the prey was transfixed and drawn into the mouth cavity. During a 24-hour period, a nymph of *B. pratense* consumed an average of 66 fourth-instar *An. subpictus* larvae released in a water bowl containing 3 L of water. The average larval consumption at different hours is presented in Table 1. One of 60 mosquito larvae consumed (average of three

TABLE 1

Average Consumption of Fourth-Instar *Anopheles subpictus* Larvae Over 24 Hours by *Brachytron pratense* Nymph*

Number of Larvae Consumed During 3-Hour Intervals								Mean ± Standard Error
Lights-on Phase				Lights-off Phase				
5:00 to 8:00	8:00 to 11:00	11:00 to 14:00	14:00 to 17:00	17:00 to 20:00	20:00 to 23:00	23:00 to 2:00	2:00 to 5:00	
5 ± 0.43	12 ± 0.62	19 ± 0.65	11 ± 0.71	9 ± 0.76	5 ± 0.75	2 ± 0.34	3 ± 0.56	8.25 ± 1.66

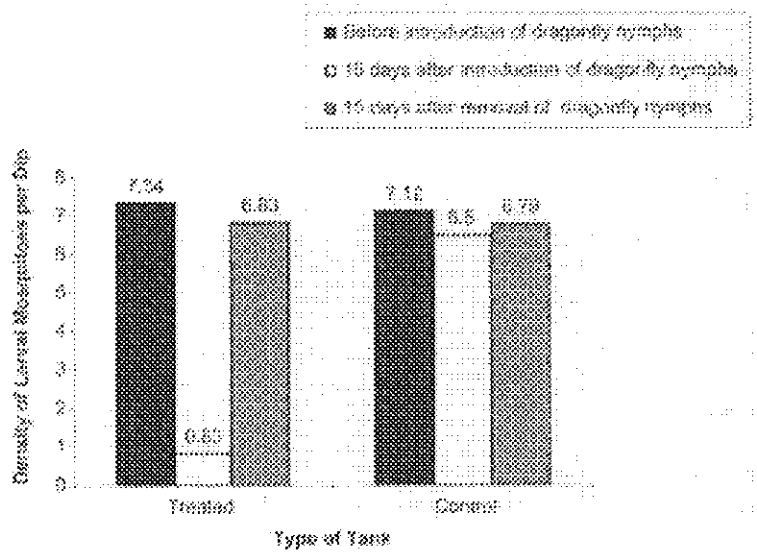
*n = 3

experiments) by a single *B. pratense* nymph in the course of 24 hours, 47 were consumed during the day (the lights-on phase) and 19 during the night (the lights-off phase). The consumption rate during daylight was significantly higher ($p < .05$) than during the night ($t = 2.155$). The reason for this difference is that the nymphs are daylight walkers—that is, they need light for visual prey acquisition.

Under field conditions, the per-dip larval density of mosquito was higher in Kalna (7.34) than in Burdwan (5.48), probably because more breeding sites are available in Kalna, which is a rural area, than in an urban slum like Burdwan. The changes in the per-dip density of a mixed population of mosquito larvae under field conditions in Kalna and Burdwan (shown in Figure 1 and Figure 2, respectively) were measured at a fixed time interval of 15 days after the introduction of 10 dragonfly nymphs in each of the treated tanks. In the nymph-treated tanks, the per-dip mean density of the mixed population of mosquito larvae was significantly reduced 15 days after the introduction of *B. pratense* nymphs ($t = 43.55$ and 35.23 in Kalna and Burdwan, respectively, in comparison with a tabulated value of 2.04, at .05 level of probability). But in the control tanks, where no nymphs were introduced, mean larval density did not differ significantly ($t = 0.79$ and 0.78 in Kalna and Burdwan, respectively). Again mean larval density increased significantly in tanks (tank numbers 1 to 5) after 15 days from the removal of the nymphs ($t = 32.29$ and 28.13 in Kalna and Burdwan respectively). The control tanks (tank numbers 6 to 10) did not show any significant difference in per-dip density, as expected ($t = 0.46$ and 0.32 in Kalna and Burdwan, respectively, compared with the tabulated value of 2.04 at .05 level of probability).

FIGURE 1

Per-Dip Larval Density in Treated and Control Tanks Under Field Conditions in Kalna (10 Nymphs per Field Tank)



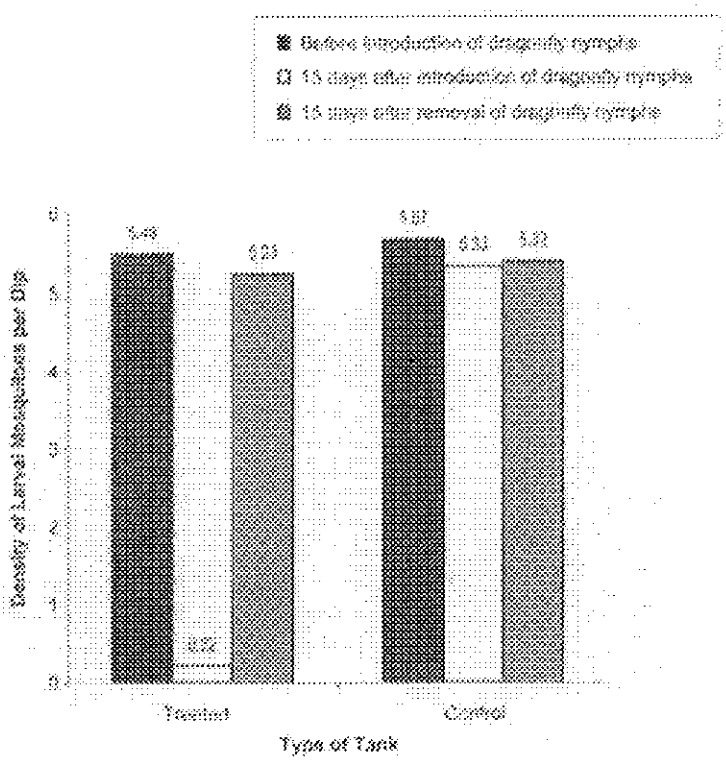
Discussion

Resistance to chemical insecticides during the late 1950s resulted in an expected turn toward a search for biocontrol agents against the mosquitoes associated with public health hazards. Jenkins (1964) reviewed the literature related to the use of natural enemies against all arthropods of medical importance, and by 1999 there had been a substantial increase in research into the use of biocontrol agents (Roy, 1974; Garcia & Legner, 1999; Murdoch, 1982; Sebastian, Srin, Tiru, & Corbet, 1990; Service, 1983). Several controversies, however, arose regarding the application of dragonflies: 1) dragonflies can displace native species, 2) augmented dragonfly populations can upset the ecological balance in aquatic ecosystems, 3) increased dragonfly populations can cause localized extinction through destruction of food supplies, and 4) suitable habitat conditions may not be found in all geographical locations where mosquito control is necessary. In spite of these concerns, some biological control programs were successful in controlling mosquito populations (Batra, Mittal, & Adhi, 2000; Wang, Hwang, & Lay, 1990; Wang, Chang, Wu, & Ho, 2000; Wu et al., 1987).

When mosquito control programs use biocontrol agents, it is highly desirable to have agents that will yield long-lasting control with one or few treatments or introductions so as to be cost-effective. During the study

FIGURE 2

Per-Dip Larval Density in Treated and Control Tanks Under Field Conditions in Burdwan (10 Nymphs per Field Tank)



equatorial force, the average daily rate at which *B. pratense* fed on fourth-instar *An. subpictus* larvae was very high (60 larvae per day), and it was higher than the rate associated with larvivorous fishes like *Gambusia affinis* (40 larvae per day) and *Labeo rohita* (32 larvae per day) (Chatterjee & Chandra, 1987). From both the laboratory and the field test results, it is apparent that mosquito larvae are a favorite food source for the dragonfly nymph, a result that agrees with the laboratory-based observations of Hati and Ghosh (1980) in Calcutta.

The field study indicated that the presence or absence of nymphs in these naturally mosquitogenic sites had considerable impact on larval densities. The potential for malaria control resulting from the reduction of mosquito larvae in their natural breeding sites after introduction of nymphs was demonstrated quantitatively. The finding that variations in larval density were insignificant in the control tanks during the period of experiment rules out the possibility that other factors influenced the reduction and confirms the role of the dragonfly nymph as an effective predator that decreases larval mosquito density in treated tanks. The increase in larval density that occurred in the same tanks after removal of nymphs further confirms the biocontrol efficacy of *B. pratense* nymphs.

Further work is necessary, however, to determine the proper methodology for the mass rearing and augmentative release of dragonfly nymphs that would make this biocontrol procedure feasible for widespread application. One method would be to select bodies of water known to be breeding grounds for mosquitoes, to create miniature biotopes adjacent to these bodies of water, and to artificially introduce native dragonfly nymphs into the water body. This process would make it possible to further explore the biocontrol potential of dragonfly nymphs.

Conclusion

Mosquitoes are important vectors of diseases, especially in the tropics. Resistance to chemical insecticides is a growing problem, and increasing attention is being paid to alternative control methods. Among the weapons available for use against mosquito larvae, biological control may be advantageous. The research reported here provided new information about the potential use of dragonflies as biocontrol agents in the worldwide campaign to control the insect vector of malaria. The study demonstrated that *B. pratense* nymphs are dependable predators of mosquito larvae. *B. pratense* feeds best during daylight. Its stalking habits make it an effective predator of many species of mosquitoes common to India. Because of its relatively long nymphal life (approximately one year in tropical locales and up to two years in colder climates) and its recorded daily feeding rate, *B. pratense* can be expected to consume a great number of mosquito larvae. The authors conclude that *B. pratense* can be used effectively as a strong biocontrol agent for the control of mosquitoborne diseases, as well as for control of mosquitoes that are or that may become resistant to pesticides. Additional research needs to be conducted to determine optimal methods for cost-effective commercial rearing and targeted use of the nymphs in various field conditions.

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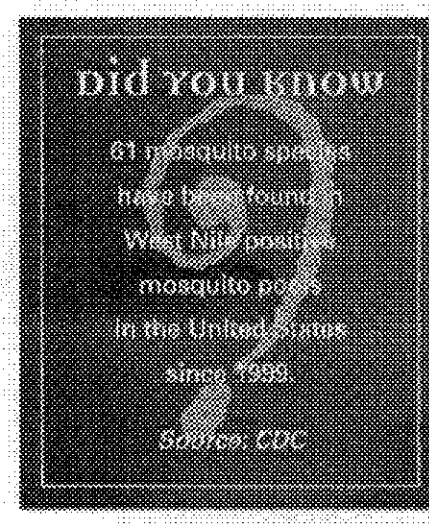

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THE EFFECTIVENESS OF THE MOSQUITO MAGNET® TRAP FOR REDUCING BITING MIDGE (DIPTERA: CERATOPOGONIDAE) POPULATIONS IN COASTAL RESIDENTIAL BACKYARDS

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ABSTRACT. This study evaluated the effectiveness of single Mosquito Magnet® traps to consistently reduce biting midge populations in a coastal northwest Florida residential neighborhood. Midge abundance from 5 backyards, each with a Mosquito Magnet trap, was compared with 3 backyards without traps (controls). Midge populations were sampled once weekly for 24 h in each backyard by using a Centers for Disease Control (CDC) suction trap baited with carbon dioxide. In addition, midge accumulations from Mosquito Magnet traps were collected once a week at the time of CDC trap operation while biting midge populations were manually sampled from each backyard by using a ''sand fly bat'' (i.e., a wire-handle flyswatter fitted with a 10.5-cm^2 plastic 14 × 14 mesh screen). Eighteen midge species were collected during the study but the majority (99.9%) collected from all backyards were (in descending order): *Culicoides mississippiensis, C. furens,* and *C. melleus.* Midge populations from CDC traps in yards with Mosquito Magnets were significantly lower on 2 of 45 wk (March 28 and April 3) when compared with control backyards. Weekly reduction attributed to Mosquito Magnets was not consistent and ranged from 4.2 to 85.3%. No significant difference was found in mean midge abundance on sand fly bats from yards with Mosquito Magnets compared with control yards. On the average, cooperators with Mosquito Magnet traps reported that their seasonal level of midge reduction (as related to a monthly ranked degree of annoyance) was variable and often not consistently below their threshold.

KEY WORDS *Culicoides mississippiensis, Culicoides furens, Culicoides melleus,* removal trap

INTRODUCTION

Biting midges (specifically, *Culicoides* spp.) continue to be a tremendous nuisance in Florida coastal communities. Reduction of midge populations through larval control by the application of pesticides to the soil, or modification of their wetland habitat, is not an option because of state and federal environmental regulatory issues. Area-wide application of pesticides to control adult midges often provides little long-term relief (Linley 1976).

Recently, a number of commercial insect traps have entered the market place that employ the concept of removal trapping as a means of control of biting flies. Although a variety of traps are available, they can be placed primarily into 2 categories, those that lure biting flies to land onto an adhesive-coated board on the trap or those that ''sweep'' the insect, by suction, into a fitted bag or net. Some of these traps employ sound or use the attractant 1-octen-3-ol to lure biting flies in. Still others use heat, moisture, carbon dioxide, or a combination of these, that are provided by the catalytic conversion of propane. Although many manufacturers claim their traps can remove mosquitoes and other biting insects from a delimited area, no published scientific data exist on the field efficacy of these traps. In response to requests by the general public for information on the effectiveness of propane-powered suction traps for the control of *Culicoides* we evaluated the Mosquito Magnet Pro® (MM) in several Florida coastal residential backyards.

MATERIALS AND METHODS

The study area was located in a residential neighborhood adjacent to an extensive *Juncus roemerianus* Scheele marsh in Panama City, Bay County, FL. This brackish tidal marsh serves as a production source of *Culicoides* spp. for this area. Eight backyards (approximately 33 m wide by 25 m deep) with very little understory vegetation and few trees that faced Callaway Bayou were used. Each residence shared a common street-front entrance. One MM (American Biophysics, East Greenwich, RI) was placed in each of 5 backyards according to manufacturer's suggested placement, that is, traps located upwind of prevailing winds in shaded, open areas approximately 9–12 m from human traffic areas. Each MM was powered by propane (provided by 7.7-kg [17-lb] pressurized tanks) and contained a slow-release sachet of 1-octen-3-ol. The MMs ran continuously during the study. Propane tanks and octenol sachets were replaced about every 21 days according to manufacturer's directions. Each backyard was separated by at least 3 residential lots (i.e., 120 m) with the exception of 2 adjacent yards that each had a MM. However, the MM in those 2 latter yards were located at opposite ends of each backyard. Three backyards without MM traps in the same neighborhood served as controls. They were also separated by at least 3 residential lots as well as from yards with MMs.

Midge populations in each backyard were monitored once a week, for 24 h, by using a 6-V battery-operated Centers for Disease Control (CDC) insect suction trap (John W. Hock Co., Gaines-

ville, FL), without a light, and fitted with a 30 × 30-mesh dark olive green, heat-resistant polyester fabric collection bag. Traps were baited with carbon dioxide by using a 9-kg (20-lb) compressed gas cylinder fitted with a FLOWSET 1 pressure regulator (American Biophysics) that delivered 500 ml/min. All traps were suspended about 1.8 m above ground level from metal poles. In backyards with a MM, CDC traps were located at the opposite side of the yard (at least 30 m) to minimize trap competition. Midge accumulations in MM traps were collected once a week at the time of CDC trap operation.

Species composition and midge abundance were recorded from CDC and MM collections. If individual collections in a trap rose above 1,000 midges per sample period, at least 2 aliquots were taken from that collection and final totals adjusted accordingly by using the methods of Cilek and Kline (2002). Percent midge reduction in backyards with MM traps was determined by using the following formula from Abbott (1925):

{[mean no. midges from CDC (control) traps
− mean no. midges from CDC MM (treatment) traps]
÷ [mean no. midges from CDC (control) traps]}
× 100%

Another measurement of biting midge abundance in backyards used a ''sand fly bat'' similar to that of Giglioli et al. (1980). The bat consisted of a wire-handle flyswatter (43 cm length) with a 10.5-cm^2 plastic 14 × 14 mesh screen. Corn oil, from a prepackaged pressurized spray can, was then lightly applied to both sides of the screen. Biting midges were sampled by walking the backyard perimeter of each yard in the morning before turning on CDC traps. At that time the sand fly bat was gently waved in front of the person in a figure 8 pattern (from waist to head) so that any biting midges on the wing would be intercepted on the greasy surface of the screen. Separate bats were used for each yard. Midges were removed from the bats, for identification, by immersion in 70% ethyl alcohol. The taxonomic guide of Blanton and Wirth (1979) was used for identification. This study was conducted from April 25, 2000, and ended June 25, 2002, for a total of 45 wk.

Every 3–4 months a mail questionnaire was sent to each homeowner with a MM in their yard. Each was asked to rank their level of midge annoyance, per month, from 0 to 5 (i.e., 0 = no annoyance to 5 = severe annoyance—midge population not reduced). This allowed us to quantify the perceived seasonal biting midge annoyance from these cooperators. We did not send questionnaires to controls because we were only interested in the perception of midge reduction by MM owners relative to our own samples of midge abundance.

Statistical analyses: Mean number of biting midges in controls and treatments from CDC traps and from sand fly bat collections, per date, were subjected to analysis of variance (SAS Institute 1990) and a *t*-test was performed to determine significant ($P < 0.05$) differences between treatments and controls for each type of collection method (Sokal and Rohlf 1981).

RESULTS

Generally, biting midges from CDC traps peaked during spring (March 15) and again in the fall (November 27) in treatment and control yards (Fig. 1a). Populations were generally at their lowest from mid-May through September. Although 18 species of biting midges were collected from CDC and MM traps, *C. mississippiensis* Hoffman (92.9%), *C. furens* (Poey) (5.0%), and *C. melleus* (Coquillett) (2.0%) comprised the majority of collections (Table 1).

Mosquito Magnets collected a tremendous amount of midges but mean reduction in backyards with these traps was sporadic, ranging from 4.2 to 85.3% (Fig. 1b). However, reduction was significantly lower on only 2 dates (March 28 and April 3) when compared with control backyards (Fig. 1a).

Average weekly midge abundance from the sand fly bat, and monthly annoyance ranking of homeowners with MM traps, generally followed similar trends as previously observed with the CDC traps (Fig. 1c). Midge abundance from sand fly bats was not significantly different in treatment yards compared with controls. Monthly ranked mean homeowner annoyance was at its lowest (≤1.0) during July and September when seasonal midge populations were minimal.

No consistent differences in the above trends from pooled CDC trap or sand fly bat data were observed when *C. mississippiensis*, *C. furens*, and *C. melleus* were separately compared between MM and control backyards.

DISCUSSION

Removal trapping is not a new concept but it has received renewed attention with the recent introduction of a variety of commercial traps marketed for this purpose. In our study, a single MM trap in a backyard adjacent to developmental habitats of larval biting midges did not consistently reduce adult midge populations below background (control) levels. Even the 2 adjacent yards with MMs did not reduce midges more than single units. We agree with Day et al. (2001), and have shown in previous work (Cilek et al. 2003), that trap systems can be periodically overwhelmed by the sheer abundance of adult host-seeking midges moving into a target zone. Although midge reduction was variable, often it was not consistently great enough for homeowners to notice a difference in their backyard. Reduction may need to be

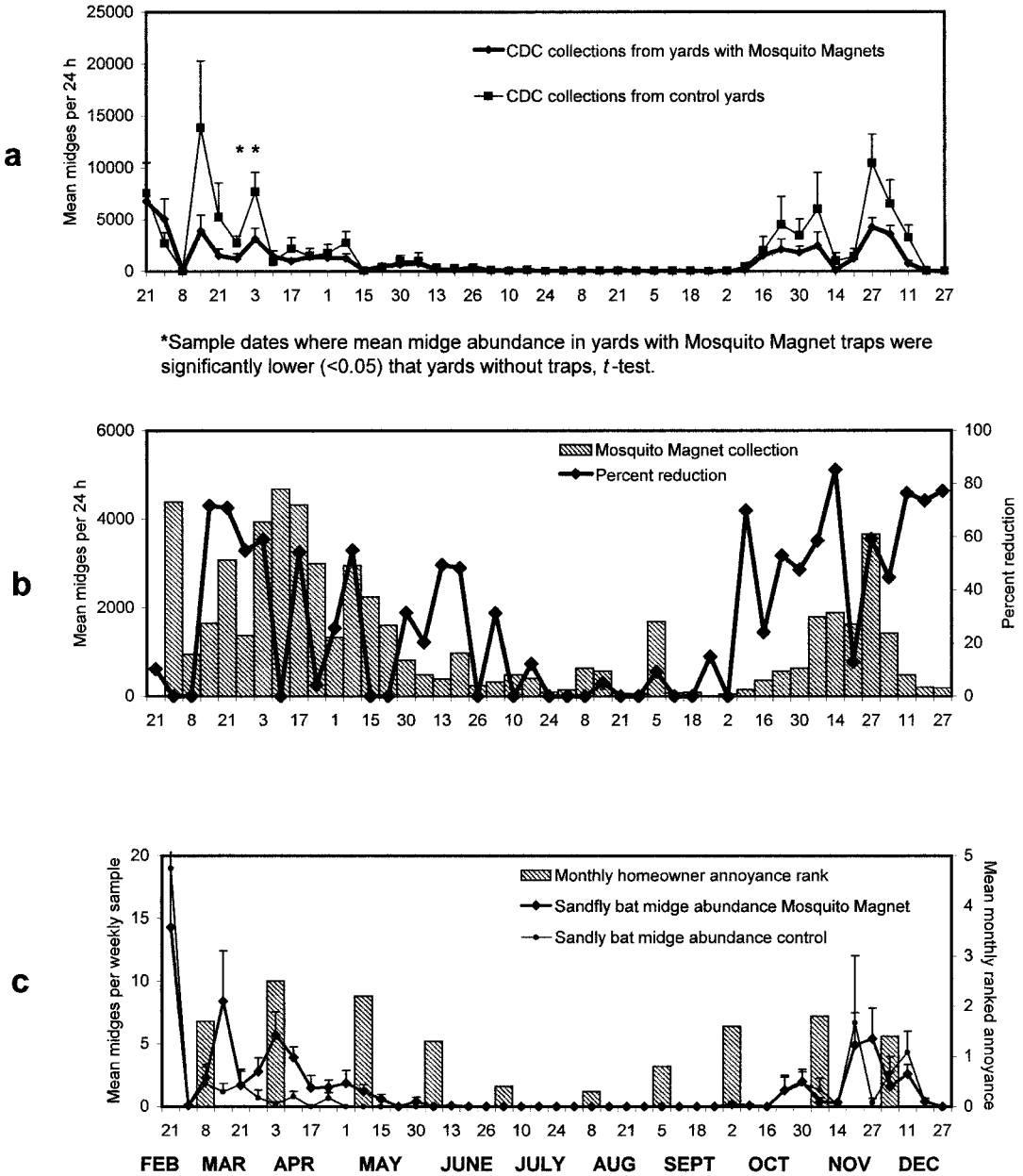

Fig. 1. (a) Mean biting midge abundance from Centers for Disease Control suction traps in backyards with and without Mosquito Magnet traps, (b) mean abundance from Mosquito Magnet traps and percent reduction in backyards with Mosquito Magnet traps, and (c) mean abundance from sand fly bat collections in backyards with and without Mosquito Magnet traps and mean monthly ranked homeowner annoyance.

obtained at a level of 95%, or greater, daily. Interestingly, when CDC trap abundance was ≤125 midges/night (i.e., during summer months), average annoyance, as reported by MM owners, was at its lowest (i.e., ≤1.0). Moreover, as midge abundance rose above this ''threshold,'' trap owners annoyance similarly increased. Indeed, Cilek et al. (2003) reported in a similar study that biting midge reduction in backyards occurred more consistently when populations were lowered to <200 midges per night.

Unlocking the key to reduction in adult biting midges by using removal trap technology remains complex and challenging. Although single traps in solitary backyards did not achieve a sufficient level of midge reduction, other placement strategies (e.g.,

Table 1. Biting midge species collected from Centers for Disease Control suction traps and Mosquito Magnet traps from April 2000 to June 2002, Callaway, Bay County, FL.

Species
Culicoides arboricola Root and Hoffman
Culicoides biguttatus (Coquillett)
Culicoides crepuscularis Malloch
Culicoides floridensis Beck
Culicoides furens (Poey)
Culicoides guttipennis Coquillett
Culicoides haematopotus Malloch
Culicoides melleus (Coquillett)
Culicoides mississippiensis Hoffman
Culicoides mulrennani Beck
Culicoides niger Root and Hoffman
Culicoides paraensis (Goeldi)
Culicoides scanloni Wirth and Hubert
Culicoides snowi Wirth and Jones
Culicoides spinosus Root and Hoffman
Culicoides stellifer Coquillett
Culicoides tissoti Wirth and Blanton
Culicoides travisi Vargas

using several traps arranged along a perimeter as a barrier) may achieve greater impact on populations of these biting pests.

ACKNOWLEDGMENTS

Appreciation is extended to the residents of Callaway Bayou, in Panama City, for their willingness to participate in this study. We also thank Mary Ann Olson, Jamie Coughlin, and Ronald Gaskill for their assistance with data collection and identification of biting midges. Special appreciation is extended to W. L. Grogan, Salisbury State University, Salisbury, MD, for identification and confirmation of several *Culicoides* species. Voucher specimens were deposited in the Entomology Collection of Florida A&M University. This project was partially funded by State of Florida, Department of Agriculture and Consumer Services contract 5895.

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EVALUATION OF AN AUTOMATIC-TIMED INSECTICIDE APPLICATION SYSTEM FOR BACKYARD MOSQUITO CONTROL

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ABSTRACT. Several manufacturers and pest management companies have begun to market and install outdoor automatically timed insecticide application systems that claim to provide an envelope of protection against host-seeking mosquitoes within a defined area, e.g., residential backyards. A typical system consists of a multi-gallon reservoir attached to a continuous loop of plastic tubing with multiple single spray head nozzles. Nozzles are usually placed along the perimeter of a backyard in landscaping or other areas suitable for mosquito harborage. This array is then connected to a programmable electric pump set to automatically apply an insecticide at predetermined intervals. An operational field study was conducted to evaluate this technology using previously installed MistAway© systems at 3 residences in northwestern Florida. This system applied a mist-like application of 0.05% AI synergized pyrethrins for 45 sec at dawn and again at dusk in each backyard. Twice-weekly collections from ABC suction light traps, baited with carbon dioxide, were used as the evaluation tool. Female mosquitoes from treatment backyards were compared with trap collections from 3 backyards without automatic misting systems used as controls. We found that weekly mosquito reduction was highly variable and ranged from 98% to 14% during the 35-wk study. Because the primary method of reduction by these application systems was not well understood, a MistAway system was installed in an outdoor simulated residential backyard to determine exposure pathway under controlled conditions with field cage and excised-leaf bioassays. Using laboratory-reared females of *Aedes albopictus* and *Culex quinquefasciatus* in those assays, we found that reduction by the MistAway system was primarily achieved by direct exposure of the mosquitoes to the insecticide application and not from residual deposits on treated vegetation.

KEY WORDS Automatic mist systems, synergized pyrethrins, threshold, *Aedes albopictus*, *Culex quinquefasciatus*

INTRODUCTION

A number of manufacturers and pest management companies have begun to market and install outdoor automatically timed insecticide application systems in order to provide an envelope of protection against host-seeking mosquitoes within a defined area, e.g., residential backyards. The marketing niche for this technology has been fueled by the desire of homeowners to enjoy the outdoors without the annoyance of being exposed to host-seeking mosquitoes, including the possibility of exposure to mosquitoes infected with an arbovirus such as West Nile.

Automated insecticide application systems for flying insect control are not new and have been used in dairy barns, equine stables, poultry houses, and beef cattle loafing areas for decades (McPhee and Hirst 1988, Sheppard et al. 1989, Meyer et al. 1990). A typical automated system for residential mosquito control consists of a multi-gallon reservoir, primarily containing synergized pyrethrins, connected to a continuous loop of plastic tubing and multiple single head spray nozzles. The system is driven by a programmable electric pump that automatically applies the insecticide at predetermined intervals. Generally, the nozzles are placed along the perimeter of the backyard (≈0.9–1.2 m above ground surface) in landscaping or other areas of mosquito harborage. Application is usually performed during periods of peak mosquito movement, i.e., dawn and dusk. In some units, application frequency can be manually increased by the end user via a remote override.

The Florida mosquito control community has questioned the effectiveness of automatically timed insecticide application systems for the control of adult mosquitoes (Rutledge-Connelly 2006). Moreover, no studies are available that sufficiently document their effectiveness (USEPA 2007). Therefore, an operational field study was conducted to evaluate this technology at 3 residences in northwestern Florida. Although it has generally been established that the exposure pathway of a space spray is by direct contact with the flying target, the primary pathway of control is not completely straightforward for these systems. The advertising literature from several manufacturers of automatically timed insecticide application systems state that continued control can be achieved when mosquitoes land on the surrounding treated vegetation after initial application. As a result, we conducted studies to determine whether the primary pathway of control was due to direct contact via inertial impaction of the spray and/or residual tarsal contact from treated vegetation under controlled conditions using a simulated residential backyard.

Fig. 1. Partial view of simulated backyard framed by a 1.2-m-high polyvinyl chloride pipe perimeter "fence" with potted wax myrtle bushes. In the foreground are cylindrical wire mesh cages suspended from wooden stakes used for the wire cage bioassays. Inset shows 1 of the 18 Hago spray nozzles that applied synergized pyrethrins from a Model Gen 1.2 MistAway® automatic-timed misting system.

MATERIALS AND METHODS

Simulated backyard study

Wire cage bioassays: To determine the exposure pathway of the insecticide application by the automatic-timed insecticide application system, a simulated typical residential backyard (15.2 m deep × 22.9 m wide) was constructed on the grounds of the Mulrennan Public Health Entomology Research and Education Center, Panama City, FL. The backyard was framed by a 1.2-m (height) polyvinyl chloride (PVC) pipe perimeter "fence" arranged in the shape of an open rectangular "U" (Fig. 1). A MistAway® automatic misting system (Model Gen 1.2; MistAway Systems, Inc., Houston, TX) was professionally installed in the backyard by a licensed/certified local pest control company to operational specifications for a residential backyard. The continuous loop system consisted of 0.5-cm-diam plastic tubing connected to 18 non-drip Hago #4023 nozzles (Hago Manufacturing Co. Inc., Mountainside, NJ) spaced 3.1 m (10 ft) apart positioned on the inner top edge of the fence. Nozzles were oriented upwards at ≈45°. A single line of 13.7-liter potted wax myrtle (*Myrica cerifera* L.) plants was placed along the inside perimeter of the PVC fence (total 85 plants) to simulate the vegetative border of a suburban backyard (Fig. 1). Tops of the plants were ≈15.2 cm below the spray nozzles. The application system was attached to a 250-liter drum reservoir that contained 0.05% AI solution of Summerfrost® (MistAway Systems), a water-soluble product that consisted of 3% AI pyrethrins, 6% AI piperonyl butoxide, and 10% AI n-octyl bicycloheptene dicarboximide (MGK 264). According to the manufacturer, system flow rate was 41 ml/min per nozzle at 180 psi.

Mosquito bioassays consisted of 14 × 14 mesh vertical cylindrical copper wire cages that measured 12 cm diam × 13 cm length with solid bottoms that contained a 1.8-cm-diam hole to load mosquitoes (Fig. 1). This configuration was used because it allowed insecticide to drift through the cages from above and sides (Barber et al. 2008). Cages were placed ≈1.5 m from ground surface on wooden stakes arranged in a grid starting 3.1 m away from the nozzles (42 cages total). At least 15 laboratory-reared 5- to 7-day-old female *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say were mouth aspirated into each cage. These 2 species were used because of their importance as possible disease vectors. Tests were repeated on 4 different calendar dates, with each species in a separate cage but tested side by side and conducted at dusk. Application consisted of a standard residential application time of 45 sec. Ten minutes after application (to allow enough time for the spray cloud to pass through the backyard) cages were removed from the treatment area. At that time, mosquitoes were removed from the cages by lightly knocking them down with carbon dioxide and transferred to clean 0.6-liter paper containers covered with fine screen cloth. A cotton ball soaked in a 10% aqueous solution of table sugar was placed on the top of each container. Knockdown/mortality was assessed at 24 h. Three untreated cages of each species were used as controls for each test and similarly processed as treatments. Temperature, RH, and wind speed and direction were recorded during each test.

Also, the droplet spectrum in front of Hago nozzles connected directly to a MistAway unit was determined in a wind tunnel using a Malvern laser by Jonathan Hornby (Lee County Mosquito Control District, Fort Myers, FL) using the methods of Hornby et al. (2006). Measurements were replicated 3 times.

Excised-leaf bioassays: Potted wax myrtle plants, previously placed along the inner perimeter of the PVC fence-simulated backyard, were used in this portion of the study. Excised-leaf bioassays were performed about 30 min after a 45-sec spray application by removing 2 adjacent leaves from the top canopy of 10 plants in the immediate vicinity of the nozzles. Single leaves were placed in individual screened 250-ml glass beakers. At least 15 female *Ae. albopictus* and *Cx. quinquefasciatus* were mouth aspirated into separate beakers. A cotton ball soaked in a 10% aqueous solution of table sugar was placed on the top of each container. Knockdown/mortality was assessed at 24 h. Tests were repeated on 7 calendar dates and both species were tested at the same time. Mean surface area of leaves used in testing averaged 11.5 ± 2.1 cm^2.

Because pyrethrins have been reported to have some repellency, the resting and landing behavior of each mosquito species in the leaf bioassays was observed at 1 h and 24 h. Repellency was considered present if mosquitoes in the treatment beakers avoided continuous contact with the treated leaves.

Residential operational field study

This study used the MistAway automatic misting system (Model Gen 1.2; MistAway Systems) professionally installed in 3 northwestern Florida backyards (yards averaged ≈17 m deep × 26 m wide) prior to this study by a licensed/certified local pest control operator. Number of nozzles in each backyard ranged from 26 to 43 (placed ≈0.9–1.2 m above ground surface) and were oriented upwards at ≈45°. All systems were programmed to automatically apply a 45-sec spray of 0.05% AI solution of Summerfrost at dawn and another application at dusk. The display panel on the unit could also be accessed to determine if the homeowner had overridden the system for additional sprays. No additional applications were noted at each treatment site during the study.

Each treatment backyard was paired with an untreated (control) yard but separated by at least 30.5 m from one another. Control backyards did not have the automatic misting system installed in them but were similar as much as possible in size and vegetation to treatment backyards. Two of the treatment/control backyard pairs were located in Bay County and the other pair was located in adjacent southern Walton County. Each paired treatment/control site was ≈32 km from one another. Approximately midway through the project (July 16; week 18), one of the treatment yards in Bay County ended their participation, leaving 2 treatment yards and 2 control yards for the remainder of the study.

Female mosquito populations were monitored in each backyard with one ABC suction trap (Clarke Mosquito Products, Roselle, IL), with the light on powered by a 6-V gel battery, per yard. All traps were baited with carbon dioxide dispensed from a 9.1-kg (20-lb) pressurized cylinder at a release rate of 500 ml/min. Traps in treatments and controls were located near the backyard perimeter. Twenty-four-hour collections were obtained twice per week, and trap collections were identified to species with the taxonomic key of Darsie and Morris (2003). The 35-wk study started on March 22 and continued through November 16, 2007. The project ended in mid-November because of the onset of cooler weather.

Data analyses

Weekly mosquito abundance from ABC traps were pooled separately for controls and treatments. Trap data were transformed using $\log\sqrt{x+1}$ and subjected to ANOVA and Wilcoxon rank-sum test (Ott 1977, SAS Institute 2002). Mean differences were considered significant at $P \leq 0.05$ and ≤ 0.10. Weekly mean percent reduction was calculated for pooled trap data from treatment yards.

An additional evaluation measure was used for determining effectiveness of the MistAway system in backyards. Weekly mean light-trap data from treatment yards were compared with an established annoyance threshold previously determined by the Florida Department of Agriculture and Consumer Services. This threshold, cited in Florida Statute 5E-13.036, stated that 25 mosquitoes, or more, per night in a light trap justified the application of an adulticide by Florida mosquito control programs (Florida Administrative Code 2006).

Mean knockdown/mortality data from cage bioassays in the simulated backyard study were transformed via $\log\sqrt{x+1}$ and subjected to an ANOVA and the *t*-test to determine differences between species within nozzle distance ($P \leq 0.05$ and ≤ 0.10) and between distance within species using the Student–Newman–Keuls test ($P \leq 0.05$) (Ott 1977, Sokal and Rohlf 1981, SAS Institute 2002). Mean percent knockdown/mortality in excised-leaf bioassays were calculated after correction for natural mortality using the formula by Abbott (1925). Means of untransformed data are presented in all tables.

RESULTS

Simulated backyard study

Wire cage bioassays: Generally, efficacy of the insecticide application against caged mosquitoes was influenced by distance from the nozzle, i.e., mosquitoes farther from the nozzle generally exhibited less knockdown/mortality than mosquitoes closer to the nozzle (Table 1). Knockdown/mortality was significantly greater at 3 and 6 m for *Ae. albopictus* but highly variable for *Cx. quinquefasciatus.* Directly comparing the toxicity to both species at each distance revealed that knockdown/mortality of *Ae. albopictus* was significantly greater to the synergized pyrethrins application at 3, 15, and 18 m compared with *Cx. quinquefasciatus*. Wind speed during testing ranged from 3.2 to 6.4 km/h. Mean and associated confidence limits (CL) of the droplet spectrum from the spray directly in front of the nozzles, as determined by the Malvern laser, was as follows: $Dv_{0.1}$ 26.9 μm (24.8–29.0 μm), $Dv_{0.5}$ 50.1 μm (47.3–52.9 μm), and $Dv_{0.9}$ 100.1 μm (92.8–107.4 μm).

Excised-leaf bioassays: Mean percent knockdown/mortality of *Ae. albopictus* exposed to treated leaves (22.3 ± 4.5%) was greater than

Table 1. Mean percent (± SE) knockdown/mortality at 24 h of caged female *Aedes albopictus* and *Culex quinquefasciatus* at various distances from treatment nozzles exposed to a 45-sec application of 0.05% synergized pyrethrins from the MistAway® automatic-timed misting system in a simulated backyard.[1]

Distance from nozzle (m)	*Aedes albopictus*	*Culex quinquefasciatus*
3	91.2 ± 2.1A	65.2 ± 11.5A[2]
6	89.4 ± 4.8A	67.6 ± 12.1A
9	44.9 ± 9.0B	42.3 ± 12.2AB
12	34.3 ± 10.3B	33.3 ± 7.8AB
15	46.4 ± 9.4B	7.3 ± 3.38B[2,3]
18	36.4 ± 10.0B	12.8 ± 5.0B[2,3]
21	24.3 ± 5.6B	33.8 ± 8.3AB

[1] Treatment means in each column significantly different ($P \leq 0.05$), Student–Newman–Keuls test.
[2] Treatment means in each row significantly different ($P \leq 0.05$).
[3] ($P \leq 0.10$); *t*-test.

Cx. quinquefasciatus (9.7 ± 3.7%) but far less than the cage bioassays. Repellency was not observed in the leaf bioassays.

Residential operational field study

During the 35-wk study, 15 and 17 mosquito species were collected from the treatment and control areas, respectively (Table 2). The 3 major pest species, in decreasing abundance, from both areas were *Aedes taeniorhynchus* (Weidemann), *Anopheles crucians* complex, and *Cx. salinarius* Coquillett. During the first 3 wk of the study, mean weekly mosquito abundance in treated yards was significantly lower compared with control yards, with reduction ranging from 98% to 71% (Fig. 2). From week 5, and through most of the summer into September 21 (week 26), mosquito populations remained relatively low due to drought conditions. At that time, populations generally remained below the State of Florida action threshold of 25 mosquitoes per trap-night in treatment and control yards. On weeks 9, 12, and 17, the mean number of mosquitoes in ABC traps were significantly lower in treatments compared with controls ($P \leq 0.10$). Consistent and substantial rainfall started again in late September (week 27), with concomitant increases in mosquito abundance. After week 27, the number of mosquitoes in treatment traps with the misting system was significantly lower for 4 out of the 8 remaining weeks, at which time mosquito reduction ranged from 91% to 48%. Also during that time, mosquito annoyance was at or above the State threshold for 2 of those weeks.

Generally, mean mosquito abundance in treated yards remained below the threshold of 25 mosquitoes per trap-night for 31 wk of the 35-wk study while mean abundance in control yards was below this same threshold for 17 wk (Fig. 2).

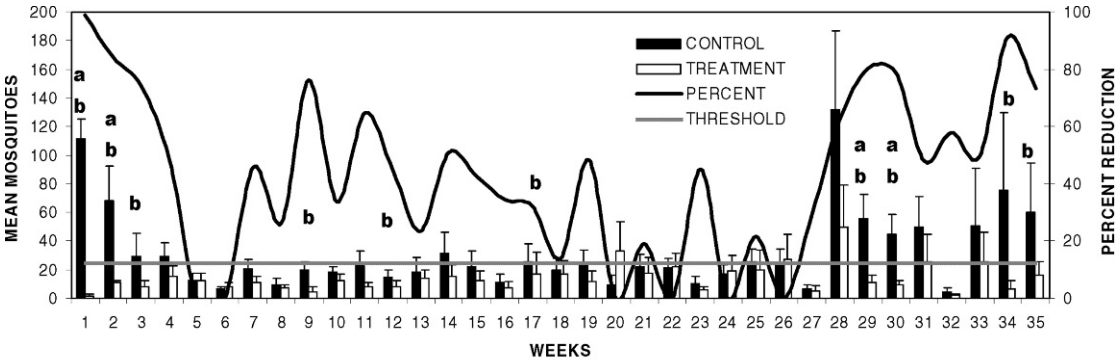
Table 2. List of mosquito species and percent collection from ABC suction light traps collected in residential backyards with and without the automatic-timed mist application system in northwestern Florida, March–November, 2007.

Species	Percent collection Treatment	Percent collection Control
Aedes albopictus	0.02	<0.01
Ae. atlanticus	0.01	<0.01
Ae. canadensis canadensis	0.07	0.11
Ae. sollicitans	0.01	<0.01
Ae. taeniorhynchus	0.20	0.24
Ae. vexans	0.05	0.10
Anopheles crucians complex	0.24	0.22
Culex erraticus	0.11	0.07
Cx. nigripalpus	0.02	0.01
Cx. quinquefasciatus	<0.01	<0.01
Cx. salinarius	0.21	0.20
Culiseta inornata	0.03	0.01
Coquillettida perturbans	0.03	0.02
Psorophora ciliata	0	<0.01
Ps. columbiae	0.20	0.03
Ps. ferox	<0.01	<0.01
Uranotaenia sapphirina	0	<0.01
Total number of specimens	2,378	5,027

DISCUSSION

Primarily, mosquito reduction in backyards with the MistAway system was achieved by the direct exposure of the mosquitoes to the spray application. Control was not consistent from week to week but fluctuated considerably and was probably influenced greatly by droplet size. Indeed, we found that knockdown/mortality in caged bioassays dropped off considerably at 9 m to <50% in our simulated backyard. Although wind speed is an important factor in drift, one cannot discount the influence that droplet size has on movement and impingement on a target, with smaller droplets traveling farther than larger ones. The droplet size range of a spray cloud for most adulticides applied by ground ultra-low volume equipment is much smaller (between 5 and 25 μm) than that from the Hago nozzles used in the MistAway system. This range is considered most efficient for impinging on a mosquito (Haile et al. 1982).

Little residual toxicity (<25%) occurred to mosquitoes exposed to treated leaves of the upper canopy after mist application and was not considered to be the primary means of reduction. Typical median droplet size distribution for an effective residual insecticide application on vegetation is between 100 and 150 μm (J. Barber, personal communication). Because of the smaller droplet size emitted from the Hago nozzles, it is plausible that not enough insecticide had been deposited on the plants to be considered as a useful residual application. Also, residual sprays are commonly applied in considerably greater



[a]Treatment and control means at each date significantly different ($P \leq 0.05$), [b]($P \leq 0.10$), Wilcoxon rank sum test. Wks 1-17 n=3, wks 18-36 n=2.

Fig. 2. Mean mosquito abundance in residential backyards from carbon dioxide-baited ABC suction light traps with and without the MistAway® automatic-timed misting system in northwestern Florida, March 22 through November 16, 2007.

volume than the 41 ml/min per nozzle in our study. Furthermore, after mist application we found no knockdown/mortality of either mosquito species when exposed to excised leaves from mid- and lower plant canopies (J. Cilek, unpublished data). It is obvious that spray volume and droplet size influenced these results.

During the summer and early fall, mosquito abundance generally remained below the State of Florida treatment threshold in ABC traps from control as well as treatment yards. This also coincided with a period of little rainfall. It is conceivable that the insecticide misting systems could have been turned off during that time and achieved similar results. This emphasizes an issue that concerns mosquito control professionals, i.e., application of insecticides on a calendar basis without regard to pest population levels. Such practices are inconsistent with integrated pest management practices. Indeed, the American Mosquito Control Association has issued a position paper on automatically timed mosquito misting systems, echoing this concern (AMCA 2008).

Additional areas of concern to be addressed are 1) the nontarget effects from organisms exposed to daily automated insecticide applications of synergized pyrethrins remain relatively unknown; 2) the effects on human health from inhalation exposure to the active ingredients in the direct spray; 3) the effects of chemical trespass into adjacent untreated areas, especially as these systems become more prevalent in residential areas; and 4) do automatic misting systems contribute to the emergence of insecticide resistance?

Individual misting units in relatively few backyards that are located in large neighborhoods are probably of little concern. But the prevalence of these systems is likely to increase. There are recent instances in which contractors who are building a few of the new housing developments in the South have installed permanent plumbing for individual backyard automatic misting systems as an incentive package for potential homebuyers.

Finally, from an operational standpoint it is unknown whether the label amount of active ingredient per acre per year could be exceeded in a neighborhood when one adds the 2 daily automatic mist applications from one (or several) backyard(s) to those of an organized mosquito control program's periodic applications of the same adulticide for area-wide control.

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ORIGINAL PAPER

Wetlands and mosquitoes: a review

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Abstract This review brings together information on mosquitoes, the diseases they transmit and the wetlands that provide habitats for the immature stages (eggs and larvae). Wetland values are mentioned, though the main literature on this does not generally overlap the mosquito issue. Mosquito management is overviewed to include: the use of larvicides, source reduction in intertidal wetlands and management in freshwater systems. There is not a great deal of information on mosquitoes and freshwater systems, except for constructed wetlands and they are considered separately. We then consider restoration mainly in the context of wetlands that have been the subject of habitat modification for mosquito control. Land use and climate change, as they affect mosquitoes and the diseases they transmit, are also reviewed, as this will affect wetlands via management activities. Finally the review addresses the critical issue of balancing health, both human and environmental, in an adaptive framework. It concludes that there is a need to ensure that both mosquito and wetland management communicate and integrate to sustain wetland and human health.

Keywords Freshwater wetland · Intertidal wetland · Mosquito · Mosquito-borne disease · Mosquito control · Wetland

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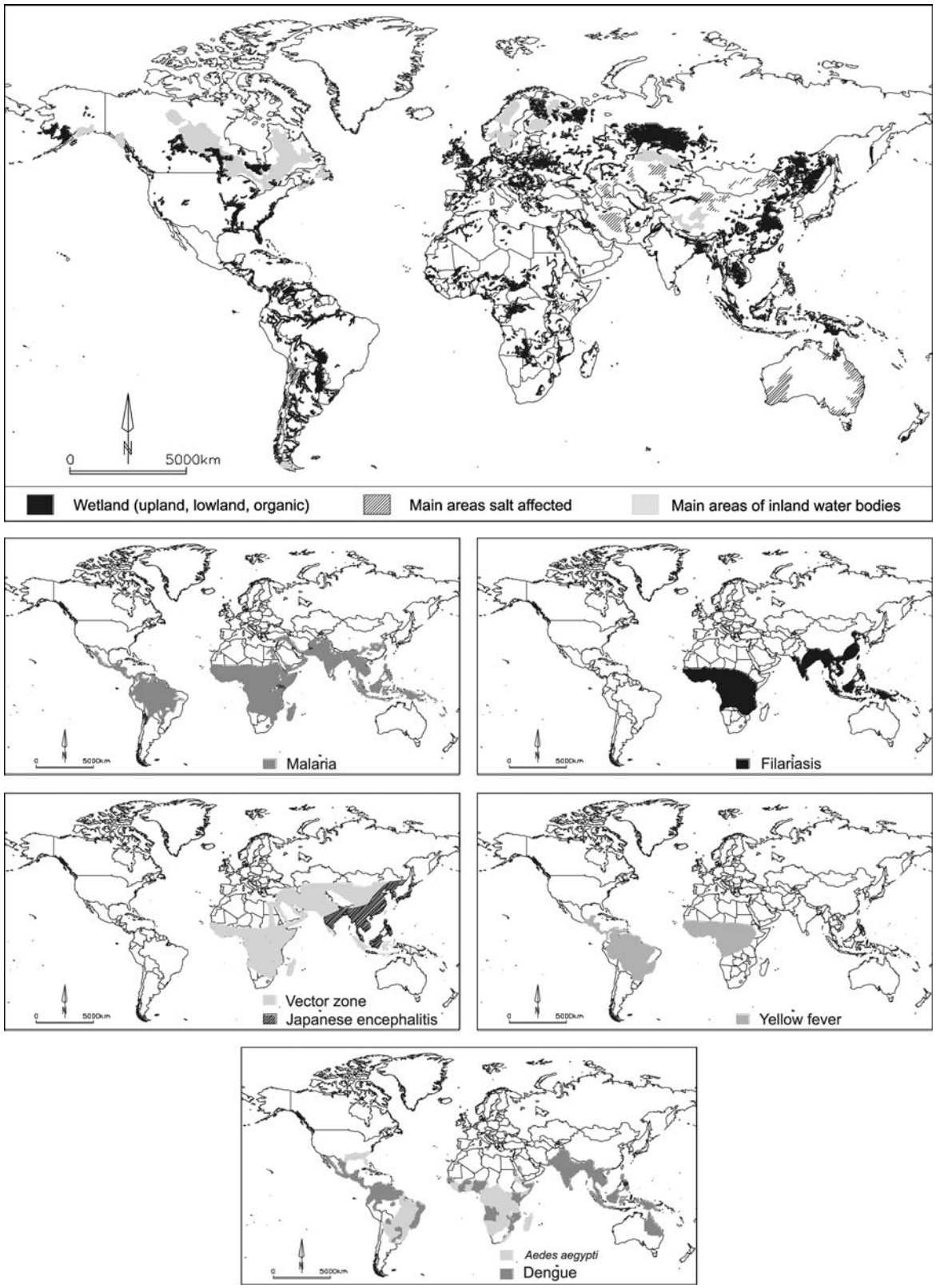
Introduction

Wetlands are indisputably important ecosystems, as evidenced by a wealth of literature spanning several decades. Many conferences, books and papers have been devoted to the subject of wetlands: their importance, threats to their integrity and management issues. There is also an extensive literature on mosquito-borne disease, mosquitoes and their management. The immature stages of mosquitoes need water and so there is a coincidence of wetlands and mosquito-borne disease vectors at both the global and local scales. To illustrate this at the global scale Fig. 1 shows the world distribution of wetlands of various kinds and the distribution of some of the major mosquito-borne diseases and their vectors: malaria, yellow fever, dengue, filariasis and Japanese encephalitis. At the scale presented, details are not shown for the salt-affected or inland wetlands nor are the large areas of permafrost in the northern hemisphere shown, though global warming could reduce the extent of permafrost and replace it with other wetlands.

This review focuses on the issue of wetlands, mosquitoes and mosquito-borne disease. It will first provide an overview of mosquito ecology, focussing on the habitats of the immature stages. It will then very briefly consider the wetlands of the world and their values, as these values may be impacted by mosquito management. The major mosquito-borne diseases will be reviewed including references to the


0
5000km
Wetland (upland, lowland, organic)
Main areas salt affected
Main areas of inland water bodies
Malaria
Filariasis
Vector zone
Japanese encephalitis
Yellow fever
Aedes aegypti
Dengue

◀ **Fig. 1** World distribution of wetlands and some significant mosquito-borne human diseases (Adapted from the following sources: World wetlands map http://www.soils.usda.gov/use/worldsoils/mapindex/wetlands.html. Accessed 25th October 2007; other diseases: World Health Organization (WHO) http://www.ciesin.columbia.edu/docs/001-613/001-613.html. Accessed 28th September 2007

wetland habitats that are important to them. The review will cover mosquito management and its impact on a range of wetlands, impacts of change (especially of climate change), and adaptive approaches to balancing the environment and human health issues. It will also highlight gaps in knowledge that need to be filled in order to understand the issues and optimise wetland and disease-vector mosquito management.

Approach

The topic of wetlands and mosquitoes is a large one. In order to obtain an overview of the field, as well as key references, we carried out a broad preliminary analysis of the literature. The database was compiled in Endnote v 9 and comprised searches on title, keywords and abstract content, to provide a broad view. The wide search was used to inform specific aspect of the review but frequency counts were restricted to title content, for focussed information. Databases included Amed and Healthstar (both via OVID), Pubmed, Web of Science and the results were combined to include the authors' personal Endnote records. Preliminary sorting removed duplicates and items that, although important to the area were not the focus of the current review. These included *inter alia* genetic analyses of mosquitoes (though not if also related to management), pathogens, vaccine development and clinical case studies. A database of over 3000 references resulted and was sorted by categories, such as 'mosquito' AND 'wetland', 'disease' AND 'mosquito'. We tried to include as many review papers as possible since they represent a synthesis that helps cover at least indirectly the breadth of the topic area. In the following sections we first outline results showing how the literature has changed since 1983 and how focus has shifted within sub-categories. We chose 1983 as it is convenient for searching electronic databases and, in any case, important earlier papers are referenced in the papers that we have cited here.

Mosquitoes and wetlands

The post 1983 literature shows a recent large increase in interest in mosquitoes and wetlands and highlights the increasing recognition that both aspects are important. Around 10% of the references included as terms both mosquito and wetlands of various types (e.g., salt marsh (in its various forms), mangrove, swamp etc.). Of these 21% were published recently, between 2004 and 2007. The following section summarises mosquito ecology with emphasis on the role of wetlands as mosquito habitats.

Mosquito ecology overview

Mosquitoes are arthropods (in the Phylum Arthropoda). The virus diseases they can transmit are often referred to as arboviruses (arthropod-borne viruses). Mosquitoes belong to the Class Insecta, the Order Diptera (flies) and, within the order, all mosquitoes belong to the Family group called Culicidae. Within the family there are Sub families and then Genera. Mosquito genera important to human disease transmission include *Aedes*, *Culex* and *Anopheles*. Each has distinct habitat requirements, and there are differences between species. The general pattern of mosquito development is from egg to hatch through four larval instars, to pupation and then emergence as adults. The timeframe for a complete cycle may be as short as five days in tropical and subtropical environments and there are differences between species in their development times.

All mosquitoes have an intimate relationship with wetlands. Water is an essential requirement for the larval stages. There are many reference works on mosquito ecology. An early reference work is that of Lounibos et al. (1985) covering community and population dynamics, ecology, epidemiology and also the role of genetics in the life strategies of the insect. The major reference text remains that of Service (1993) who reviewed the literature on the topic of the ecology, sampling and modelling of mosquito populations. There is also a literature that is used by mosquito control agencies, on a national or regional basis, such as the work for south east Australia by Russell (1993) that provides a concise overview of the ecology of and key to the common mosquitoes and their typical habitat characteristics.

The eggs are laid in water in rafts of multiple eggs (*Culex* spp.), on water singly (*Anopheles* spp.) or

singly on damp substrate that will later be flooded (*Aedes* spp.). In water, the eggs hatch into larvae that go through four instar stages. During this stage they feed on small organisms or decaying material. They breathe air through a siphon that is at or protrudes through the water surface, or, in some species, they attach to plant stems and obtain oxygen directly from the plant tissue. After the fourth instar they pupate and then emerge as adult flying insects. In some species the newly emerged adult female may be able to lay fertile eggs but generally a blood meal is required for protein to produce eggs. For disease vectors the pathogen is picked up during the blood meal and, if it replicates within the mosquito, it may be transmitted later to a victim during another blood meal. In some cases diseases may be passed on directly from the adult female via the egg to the larva and hence to the emerging adult. This is known as vertical transmission. From a wetland perspective it is important to identify the habitats of the immature stages (eggs and larvae) as these habitats are usually wetlands and the focus of larval, and hence wetland, management.

Oviposition sites (egg laying)

Although the egg stage determines generally the area that larvae will start their cycle, information is not detailed for all vectors of disease. Knowing where oviposition sites are is an aid to identifying larval habitats as larval survey may miss some of these, such as ephemeral sites when they are dry. Mosquitoes that have received much attention include the aedine nuisance species or vectors of viruses, such as *Aedes taeniorhynchus* (Wiedemann) in Florida and *Aedes vigilax* (Skuse) in Australia. That is, in part, because these species lay eggs on the ground surface and hence can be sampled directly (as compared to setting oviposition traps). Eggshells of *Aedes* spp. are good indicators of oviposition and can be used to sample at times when larvae are not abundant or are absent, as eggshells are relatively stable both spatially and temporally (Ritchie 1994). Eggshell studies have been carried out for open vegetation such as salt marsh, mainly in Australia (Dale et al. 1986; Kay and Jorgensen 1986; Ritchie 1994; Ritchie and Jennings 1994; Gislason and Russell 1997; Turner and Streever 1997; Dale et al. 2008) but there has been relatively little research on the more complex mangrove forest systems (Ritchie and Johnson 1991a, b; Ritchie and Addison 1992).

Mosquito larval habitats

The element common to all mosquito species is the need for water for the larval and pupal stages. Knowing the association between wetlands and mosquitoes is important for mosquito-borne disease as it helps to focus management. Rodriguez et al. (1996) found associations between landscape features including elevation and vegetation (mangroves) and malaria vectors in Mexico. Mercer et al. (2005) found significant relationships between immature mosquitoes and habitat characteristics such as water quality, in wetlands in Iowa (USA). They also explored the literature to further identify microhabitats associated with mosquitoes and used these to estimate the risk of disease transmission. Most of the published research on larval habitats of *Aedes taeniorhunchus* in mangrove forests has been by Ritchie and colleagues, for example Ritchie and Addison (1992). Although not focussing on larvae, there have been compilations of mosquito species recorded, for instance, in Indian mangrove systems (Rajavel et al. 2005a, b; Rajavel and Natarajan 2006).

At a detailed level, Sogoba et al. (2007) in Mali, for malaria vectors, found that dry season habitats provided a refuge for mosquito populations and suggested that focusing larval control on those would reduce the wet season populations. Miller et al. (2007) showed that *Anopheles gambiae* s.l. larvae can survive in wet mud as well as in water and that this explained their survival in an uncertain environment (with implications for management).

Detailed local studies of larval habitats highlight the need to efficiently identify them so as to focus management (Balling and Resh 1983). Field survey is important to confirm the larval habitat status of a site but Remote Sensing (RS) and Geographic Information Systems (GIS) can speed up the process and survey large areas cost effectively. Dale et al. (1998) reviewed some examples for disease vector mosquitoes in Australia. Other research includes Dale and Morris (1996) who used aerial photographs to identify ephemeral wetlands in urban subtropical Australia; Mushinzimana et al. (2006) who used 1 m spatial resolution satellite imagery for African highlands in Kenya and Bian et al. (2006) who used GIS

to estimate the spatial distribution of anopheline larval habitats in the highlands of West Kenya, taking into account terrain, landuse and surface water. Several studies have mapped the larval habitats of vectors of West Nile virus (WNV) in USA. For example, Diuk-Wasser et al. (2006) developed a spatial model showing that various mosquito vectors had clear habitat preferences and Zou et al. (2006) were able to distinguish larval habitats in Wyoming (USA) using Landsat imagery.

Information gaps

Detailed information on the immature stage habitats is relatively sparse in the refereed literature. Most of the local information is held, if at all, by vector control agencies. Management needs to have information at a scale relevant to the organism and that is at a fine level of detail. This information gap was noted by Vezzani et al. (2006) who saw a need to identify larval habitats of filariasis vector mosquitoes so as to focus management on relevant areas. In particular, there is lack of research on the immature stages of mosquitoes in forested wetlands, both freshwater and intertidal. Such research is inhibited by survey difficulty but there is potential to use novel RS technology (e.g., Dale et al. 2005b), though resources may limit this.

Wetlands of the world and their values

That wetlands are important and valuable is widely acknowledged. Schuyt and Brander (2004) have documented wetland types and their economic values and this is summarised at a global scale in Table 1.

According to Schuyt and Brander, North America and Latin America contain significant proportions of the world's wetlands (36% and 19% repectively) as well as having serious mosquito-borne diseases.

That wetlands are threatened by change both natural and anthropogenic has been recognised in books and compilations such as that of Mitsch (1998) who noted the importance of the world's wetlands and the processes that threaten them. A recent article in Science articulated the concern that mangrove losses threaten the ecosystem services they provide and thus the resource for future generations (Duke et al. 2007). A significant global advance in documenting the world's wetlands of international significance was made by the Ramsar convention in 1971. There was a recent overview of this and comprehensive inventory of listed wetlands as of March 2008, when 160,158,832 ha of wetlands in 1722 sites were protected by the convention (Secretariat of the Ramsar Convention 2008). The Ramsar aim is to develop and extend a global list of wetlands that are ecologically significant for maintaining biodiversity, ecological functions and for sustaining systems upon which much life depends.

In the overlap between mosquitoes and wetlands, wetland value is not often mentioned and mosquito value is itself a novel concept. Exceptions include Mercer et al. (2005) who stressed the value of wetlands and the ecological services they provide, measuring the abundance of mosquitoes as well as other insects and recording other environmental variables such as water quality and vegetation cover in freshwater reconstructed wetlands in Iowa USA. Mosquito value was also addressed by Schafer (2004) who considered the contribution that mosquitoes make to wetland biodiversity in a range of wetland types from forest to meadow to shallow water. Schafer's research is relevant to addressing a frequently asked question: What use are mosquitoes? Mokany (2007) suggested that mosquitoes, being abundant in ephemeral wetlands, can have a significant effect on ecosystem processes and functions and, by implication, wetland value.

Table 1 Global area of wetland types

Type	Mangrove	Unvegetated sediment	Salt/Brackish marsh	Freshwater marsh	Freshwater woodland	Total
Area (1000 ha)	12,112	45,788	6758	765	9657	62,967
Total value $1000US/year	185,667	2,848,575	73,382	3836	333,223	3,444,682

Source: Schuyt and Brander (2004)

A great deal of the wetland literature is in the area of ecology, hydrology and ecohydrology, with the latter as an important area that brings the first two disciplines together. However, relatively few papers also overlap with mosquitoes or their habitats although most wetlands have some potential as mosquito habitat. To manage the mosquito problem the ecohydrology of their environment needs to be understood. References that are useful in this respect for the intertidal systems in general include Elliott et al. (2007). For specific systems, the book by Mazda et al. (2007) bring together a literature that addresses mangrove processes in detail in order help conserve the systems. The review of salt marsh management by Dale and Hulsman (1990) addressed both salt marsh values and mosquito management issues. A specific study demonstrating the application of an hydrological approach is that of (Shaman et al. 2002) who used a dynamic hydrology model, based on meteorological data, topography, soils and vegetation to model wetness and thus to predict mosquitoes in New Jersey, USA. Freshwater wetlands are covered to some extent by the constructed wetland literature (reviewed below) but there appears to be little information on freshwater forested wetlands.

Information gaps

Wetland studies that consider wetland values, from a human perspective, tend to focus on the positive values and do not take much account of the negative ones. This needs to be addressed through research to inform a balanced approach to management. Having made that point, there is also an argument that intrinsic value should include the fauna in its entirety, including mosquitoes, as contributing to value, demonstrated, for example, by Schafer (2004). This again needs more research to investigate the nature of the values.

Mosquito transmitted diseases

There has been a growing literature on mosquitoes and disease, especially for WNV, after its introduction into the USA in 1999, affecting both humans and animals. Of over 1000 references to WNV in our database, 96% were dated 1999 or later. The spread of WNV prompted the review of host-vector disease models by Wonham et al. (2006). That 78% of all the references relate to WNV and diseases such as Ross River virus also highlights the preponderance of research into diseases of the developed areas of the world that have much less serious consequences for human health than, for example, malaria or filariasis. There are also general reviews for arboviruses in particular regions, such as the review of a wide range of arboviruses in western Europe by Lundstrom (1999). Vector competence (the ability to transmit disease) may be used to prioritise mosquito and wetland management and so is briefly introduced in the next section.

Vector competence

Mosquitoes may be a nuisance, but where they are also capable of transmitting disease to human (or animals), the case for direct or indirect management becomes important. Although there may be a correlation between the presence of specific mosquitoes and mosquito-borne disease, vector competence research is needed to confirm the relationship, usually by means of laboratory experiments. There is a large amount of research on this area, some of which reports correlations between specific diseases such as WNV and mosquito species (e.g., Dennett et al. 2007). Most vector competence research focuses on a specific mosquito or on a specific disease. A few recent examples are given here. For a mosquito species *Aedes aegypti* (L) has received much attention: as a vector of filariasis (Tiawsirisup and Nithiuthai 2006); of yellow fever (Johnson et al. 2002; Jupp and Kemp 2002) and of dengue (Knox et al. 2003). For a specific disease, WNV, the vector competence of several species has been explored, for example, of *Culex* spp. (Weng et al. 2000; Vaidyanathan and Scott 2007; Balenghien et al. 2007; Reisen et al. 2006), of *Culiseta* and *Culex* spp. (Reisen et al. 2006) and of Californian species including *Culex* spp., *Ochlerotatus* spp. and *Aedes vexans* (Meigen) (Goddard 2003; Chen et al. 2000). The next section provides an overview of the major diseases shown in Fig. 1 (and some others) and the wetland environments that support their vectors.

Malaria

Malaria in humans is a serious disease, endemic to the tropics, whose pathogen (*Plasmodium* spp.) is

transmitted by mosquitoes of the genus *Anopheles*. The mosquitoes occupy a wide range of habitats from freshwater to saline and these may be subdivided according to elevation, landform or land use. The literature includes area-specific research on the *Anopheles* spp. habitats. Some, that also focus on disease, are shown in Table 2 and others that focus on the mosquitoes include Stoops et al. (2007) who found general associations with higher elevation and lower tree canopy coverage, higher water temperatures, and shallow water as well as more species specific associations in Indonesia. Muturi et al. (2006) found relationships between land use and mosquito abundance (*Anopheles* and also *Culex* spp.) in Kenya. Climate, especially temperature, is important as the pathogen does not thrive in low (<16°C) or high (>32°C) temperatures. This has implications for climate change and its potential impacts on malaria distribution.

Malaria in Sub-Sahara Africa has received a great deal of attention, as it is one of the most significant diseases there. It is also significant in other places and a review of research conducted in six areas in Indonesia that examined the relationships between malaria and environment, including socio-economic variables, found spatial and temporal heterogeneity in most variables, indicating the need for locally specific information for management (Dale et al. 2005a).

Some of the genetic research also has relevance to the distribution of the disease and hence control of wetland vectors. For example a recent paper by Yawson et al. (2007) concluded that ecological barriers are more important to gene flow in *Anopheles gambiae* than geographical distance. Different genotypes may have different vector competencies, resulting in different disease incidences and hence priorities for mosquito or wetland management.

Yellow fever

Yellow fever is not well covered in the research literature, at least for material that includes wetlands. Much research appears to be in the area of genetics of its major vector *Aedes aegypti* and hence is not covered here. As well, the vectors are mainly container breeders and natural wetlands are not necessarily the preferred habitat. A useful review is that of Bourgeade and Marchou (2003) who describe the epidemiology of yellow fever in Africa and South America.

Filariasis

Filariasis is vectored by a range of mosquito species both aedine, anopheline and culicine and infects both humans and dogs. It occurs widely (Fig. 1) but is not much reported in terms of the focus of this review. Vezzani et al. (2006) reviewed its history in Argentina. Its vectors in Italy were reviewed by Cancrini et al. (2006).

Table 2 Disease, vector and distribution examples by continent/country (as far as possible references include reviews)

Disease	Main vector(s)	Continent/Country and reference
Malaria	Anopheline mosquitoes	General and landuse related (Yasuoka and Levins 2007), Africa (Githeko et al. 2006); South America, Southeast Asia (Taiwan) (Lien 1991). Papua New Guinea (Cooper et al. 2006)
Yellow fever	*Aedes* spp.	Africa, South America (Bourgeade and Marchou 2003)
Dengue	*Ae. aegypti*, *Ae. albopictus*	General (Bourgeade and Marchou 2003), South America (Mendez et al. 2006), Southeast Asia and Kenya (Hay et al. 2000)
Filariasis	*Culex* spp.	South America (Vezzani et al. 2006), Europe (Cancrini et al. 2006)
Japanese encephalitis	*Culex* spp.	General (Bourgeade and Marchou 2003; Spira 2007), Southeast Asia, Nepal (Partridge et al. 2007)
Ross River or Barmah Forest viruses	*Ae. vigilax*, *Ae. camptorhynchus*, *Culex annulirostris*	Australia (Kelly-Hope et al. 2004), Australia (Quinn et al. 2005; Russell 2002), Fiji (Klapsing et al. 2005)
West Nile virus	*Culex* spp. *Ae. aegypti*	North America (Hayes and Gubler 2006; Tyler 2004), South America (Bosch et al. 2007), Europe (Kaptoul et al. 2007), Western Europe (Lundstrom 1999), South Africa (Jupp 2001), India (Paramasivan et al. 2003)

Japanese encephalitis

Japanese encephalitis (JE) is a virus transmitted by *Culex* spp. and often associated with pigs close to human habitation. It may be included in discussion of WNV, though JE is a more serious disease, often resulting in death or permanent impairment. It is only found in Asia in a rough triangle from Pakistan to Indonesia and to Siberia (Spira 2007) though its vectors are more widespread (Fig. 1). Bourgeade and Marchou (2003) showed three zones of the disease: epidemic, to the north (e.g., Korea, Japan, Nepal, northern India), endemic to the south (Papua-New Guinea, southern India, Indonesia) and a transition zone between. There are many papers on aspects of the disease itself that also include the term mosquito but few refer to wetlands. A study by Hemmerter et al. (2007) is an example of genetic research relevant to this review. They suggested that the lack of JE in Australia may be related to a different lineage of *Culex annulirostris* (the major vector in south east Asia), that may not be a very competent vector.

West Nile virus

Since the arrival of WNV in USA in 1999 it has generated a great deal of interest. Initially it was not regarded as a very serious disease but it is now recognised as causing death, especially in the elderly (Bourgeade and Marchou 2003). Kramer et al. (2007) reviewed the disease comprehensively and included information on its ecology. Petersen and Roehrig (2001) suggested that WNV was a re-emerging global pathogen and that theme has been developed by others, including, for example, Boyer et al. (2002) who viewed WNV as the first pandemic of the twenty-first century. Others include Gerhardt (2006), Glaser (2004), Hayes et al. (2005) and Hayes and Gubler (2006).

Concern over the spread of WNV was reflected by Hubalek (2000), who reviewed its epidemiology in Europe with the aim of informing what might happen in USA after the initial detection of the virus in 1999. There have been several reviews of aspects of WNV such as its introduction and subsequent spread in USA (Gould and Fikrig 2004), its biology and potential to spread to South America, citing mosquitoes in the genus *Culex* as the major vector (Granwehr et al. 2004). Jourdain et al. (2007) referred to risk in the Camargue in southern France and Kaptoul et al. (2007) noted the first case in Spain. Risk of WNV has been assessed for the British Isles by Medlock et al. (2005), based on the ecology of the mosquito vectors.

Many of the references relate to birds: their deaths and their potential to spread the disease e.g., Medica et al. (2007), Koenig et al. (2007) and Ladeau et al. (2007). Hubalek (2000) noted that wetlands are implicated in the transmission cycle via the role of birds.

As well as indicating mosquito vectors some papers also provide information on their habitat characteristics, landscape and climate. Pecoraro et al. (2007) found relationships between WNV and landscape and climate (this is also relevant to climate change concerns, to be discussed later).

Ross River and Barmah Forest viruses

Ross River virus is important in the Asia Pacific and especially in Australia. It has been reviewed by several authors including Russell (2002) and Gatton et al. (2004). It is widely distributed, as there are several vectors whose habitats cover a range of wetland type from intertidal to freshwater and from permanent to ephemeral water bodies (Kelly-Hope et al. 2004). Muhar et al. (2000) found that risk of Ross River virus in Brisbane, Australia was significantly related to several wetland habitats including intertidal and freshwater ones associated with floodplains. More recently, Barmah Forest virus has become a public health issue in Australia, with symptoms similar to Ross River virus and the two diseases have some mosquito vectors in common (Doggett et al. 1999; Quinn et al. 2005).

Dengue

Dengue is mainly vectored by peridomestic mosquitoes, including *Aedes aegypti* (and more recently *Aedes albopictus* (Skuse)) so it is not a major focus for wetland issues. Nevertheless *Aedes albopictus* vectors other arboviruses (La Crosse, WNV), is also found in natural wetlands and has the ability to spread rapidly, as noted by Benedict et al. (2007).

Information gaps

What emerges from a review of mosquitoes and disease is that relatively little emphasis is placed on

the wetland habitats in which the vectors spend the critical larval stage of development and on which management often concentrates. The focus tends to be on the species that transmit disease and the relationship between vector and host (including intermediate hosts).

In practice, mosquito-borne disease management includes a range of actions from health education and clinical practice to wetland management with a focus on larval habitats. The next section focuses on larval management in wetlands.

Mosquito management and its impacts on wetlands

While this section focuses on mosquito management, the other side of the coin needs to be kept in mind: wetland management and how this interacts with mosquito control. An Australian perspective was provided by Dale and Morris (1994). In the present review 32% of the 430 'mosquito' AND 'management' OR 'control' references referred to wetlands and, of these, 17% related to constructed wetlands (discussed below). Reducing the risk of mosquito problems is most effectively done at the larval stages when the larvae are spatially concentrated. Adulticiding may be carried out but, by the time the adult flying stage is reached, dispersal is often several kilometres and may be up to 50 km for some species. It is important to note that larvae per se are not a human disease or nuisance problem. The problem is when they emerge as adults ready to take a blood meal and both larviciding and source reduction aim to prevent adult emergence.

There seems to be a trend away from attempting to annihilate mosquitoes or destroy the environment. Had we looked further back in time we would find that early mosquito agencies in USA often had the term 'extermination' in their name. For example, the New Jersey Mosquito Extermination Association was created in 1914 and later changed 'Extermination' to 'Control', as it now is. There is a large grey literature, including manuals for mosquito management published by various mosquito control organisations but these are not reviewed here. Some were referenced in Dale and Hulsman (1990) in the salt marsh context. They noted an evolution from an approach that may destroy the intertidal wetland as well as its mosquito population to a more benign approach that seeks to minimally alter the environment, based on the premise that relatively subtle changes may affect the mosquito without destroying wetland function.

Mosquito management methods have been developed over a lengthy period, especially in USA. Patterson (2004) reviewed the development of mosquito control in USA in his book "Mosquito Wars". In it he described the evolution from reliance on chemicals such as DDT to source reduction (habitat modification of wetlands) and to the chemicals that are currently used and that are believed to be less injurious to the environment than those used in earlier decades. Floore (2006) reviewed the history specifically of larval control in USA from the 1900s, when water bodies were treated with kerosene or an arsenical product, to today's methods of larviciding and source reduction.

In the context of disease control there is also research being conducted on ways to reduce the vector competence of mosquitoes, such as discussed in Joardar (2005), or to develop methods based on genetics, an approach reviewed by Tu and Coates (2004) and reviewed and explored by Linser et al. (2007) or to use radiation (e.g., Helinski et al. 2006). If successful and adopted they would minimise the direct impact of mosquito management on wetlands. However, none of these are yet in common use and so are not considered in the following sections.

The following will provide a general overview of current larval management methods and their impact on wetlands, to include: source reduction in intertidal wetlands (salt marsh and mangroves), freshwater and forested wetlands (not mangroves) and constructed wetlands.

Larvicides and their impacts on wetlands

Larvicides have a role to play in disease reduction. For example, Walker and Lynch (2007), in their review of larvicide use in tropical Africa, noted its effectiveness in reducing malaria, especially when used in conjunction with other methods (e.g., bednets, source reduction).

Larvicides are generally highly effective at killing mosquitoes, though resistance may become a problem. It is beyond the scope of this review to review the products used to control/manage larval populations but some general comments can be made. There is a range of chemical and biorational larvicides

currently in use. The main chemical categories include, for example, some organophosphates, surface agents such as monomolecular surface films and oils as well as products that are termed biorationals. Floore (2006) listed products that are generally no longer used in USA and reflecting the growing concern for environmental impacts. Those products included various oils, organophosphate and chlorinated hydrocarbons, though specific products varied by state. For biorationals, Floore (2007) edited a 330 page supplement to the Journal of the American Mosquito Control Association dedicated to this topic. The publication contains reviews on a wide range of biorational control agents, a few of which are referenced here. They include the widely used synthetic bacterial agents such as *Bacillus thuringiensis* var *israelensis* (*B.t.i.*), *Bacillus sphaericus* (Lacey 2007) and the insect growth regulator methoprene (Henrick 2007).

Forays into the field of biological control agents have had variable results. Other insects that are predators of mosquito larvae are reviewed by Quiroz-Martinez and Rodriguez-Castro (2007). Copepods are an example of biological organisms that predate on mosquito larvae (Marten and Reid 2007). Fish may be effective in some circumstances, though the use of *Gambusia* may have adverse effects on native fish (Walton 2007). Recent experiments with a mould (*Lagenidum*) were encouraging, including its safety for non-target organisms (Vyas et al. 2007).

Because of resistance problems there is a wealth of literature on testing new products or variants of existing ones. For example Park et al. (2005) developed a new strain of *B.t.i.* effective against vectors of WNV. In the present review over 10% of papers referred to efficacy testing of mosquito control agents but few of these also considered impacts on non-target organisms, an important aspect for wetland health. Exceptions include Vyas et al. (2007) noted above, for a mould, and Merritt et al. (2005) for *B. sphaericus*, noting no detrimental effects on, for example, taxa richness and abundance of non-target invertebrates, for six applications of insecticide over a three-year study.

Impacts

Dale and Hulsman (1990) in the context of salt marsh mosquito management noted that research on the impacts of chemicals on non-target organisms generally did not assess long-term or chronic impacts. Papers that assess the impacts of larvicides on non-target organisms may include field components associated with laboratory assessment, but long-term effects and effects on food webs are still not well understood. A study of the sub-lethal effects of selected larvicides on a native fish species was investigated by Hurst et al. (2007) and found no significant impacts for microbial larvicides and insect growth regulators, but did note effects of some organophosphate compounds. All were tested at concentrations greater than those used for mosquito management.

Source reduction and its impact on intertidal wetlands

Source reduction simply means reducing the source of mosquito populations. This does not necessarily mean destroying the wetland, as relatively small changes may adversely impact the mosquito life cycle and prevent adult emergence. In the intertidal environment the general group of source reduction methods currently in use in the USA include tidal recirculation, reported by Resh and Balling (1983) for California, Open Marsh Water Management (OMWM), first developed in New Jersey in the late 1960s (Ferrigno and Jobbins 1968), and developed on east coast USA intertidal wetlands, usually for those that had been ditched. By the 1980s, the OMWM method was widely used and guidelines for its implementation were developed and accepted by the relevant federal agencies (US Corp of Engineers and the US Fish and Wildlife Service). Other guidelines include those of Bruder (1980), Meredith et al. (1985) and Hruby and Montgomery (1986). Open Marsh Water Management was reviewed by Wolfe (1996) and its much smaller Australian version, runnelling, was reported in Hulsman et al. (1989).

In Florida, a state that was extensively impounded for mosquito control in the 1950s and 1960s, the recognition of wetland values led to the Rotational Impoundment Management (RIM) concept whereby tidal circulation is allowed during the winter when mosquitoes are less of a problem, and the impoundments are flooded up in spring to reduce the summer habitats of the immature stages (Carlson and Carroll 1983; Carlson and Vigliano 1985; Carlson 1986). The RIM method works by keeping water levels high and

consistent and so the damp-drying-wetting conditions needed by aedine mosquitoes for oviposition, egg conditioning and hatch are not satisfied. Schmalzer (1995) reviewed the history of this aspect of the Florida marshes.

Impacts

Impacts of the various methods of source reduction have been assessed, generally over the short term (<3 years). Examples include, for OMWM, early papers reported in the reviews by Dale and Hulsman (1990) and Wolfe (1996) and in the assessment by Meredith et al. (1983). Much of the early work was published in conference or meeting proceedings and is not easily accessed (e.g., Romanowski and Risch 1986). Research was at that time in response to the growing concerns about coastal wetlands, their values and threats to them or to their wildlife, such as birds (Brush et al. 1986). More recent research/monitoring is in reports such as the comprehensive assessment for Suffolk County (Wertheim Demonstration Project) north east USA (Cashin Associates 2007) and theses (Latchford 1997; Breitfuss 2003), and is generally relatively short term. For a longer term study the 20 year monitoring of runnelling in Australia is relevant (Dale 2007). Some innovative multivariate impact assesment techniques have been developed for that research examining effects on process rather than simply on the state of the system (Dale et al. 2002; Dale and Dale 2002).

The impacts of impounding have been discussed in the reviews referred to above (e.g., Dale and Hulsman 1990; Patterson 2004). Florida (USA) features largely in this area of research with impacts assessed for a variety of organisms (e.g., for fish, Harrington and Harrington 1982). Brockmeyer et al. (1997) in their paper on rehabilitation of impounded estuarine wetlands noted that impounding had had "a profoundly negative impact" on the wetlands (p. 96). A response has been to develop the RIM method (referred to above) that restores or partly restores tidal circulation to impoundments. Impact assessment of RIM has focussed on the extent to which it restores flora and fauna, but a major objective is also to manage mosquitoes (see Carlson and Vigliano 1985). One difficulty is that, after being impopunded for some decades, the marsh elevation may not have evolved in line with natural marshes, and may be relatively lower in some impoundments (Parkinson et al. 2006). They suggested that restoring tidal flooding, unless this is done very gradually, may result in water to depths that inhibit plant colonisation and hence jeopardise restoration success.

Freshwater wetlands

Research on freshwater wetland communities may note mosquitoes, but may not mention disease risk or suggest management. For example, Reese and Batzer (2007), studying the floodplain of the unregulated Altamah river in south east USA, found a diverse assemblage of aquatic fauna and mentioned rapidly developing organisms including mosquitoes associated with the upper reaches. Ephemeral wetlands may be important for mosquito populations. Certain species (such as *Culex annulirostris* in Australia) are known to rapidly colonise ephemeral wetlands. In their work on the wetlands of Iowa (USA), Mercer et al. (2005) found that the greatest risk of disease transmission was associated with ephemeral pools, an observation supported by others such as Dale and Morris (1996). Also in an ephemeral pond context, Mokany (2007) considered the impact of mosquito larvae (and tadpoles) on the invertebrate community, although without reference to potential human health impacts of the mosquitoes after emergence from the wetland.

Natural freshwater wetlands include both forested wetlands (swamps) and marshes and have been a neglected area of mosquito-specific research. Forested wetlands include habitats for mosquito vectors of disease. For example Hachiya et al. (2007) noted that the areas sampled for the Eastern Equine Encephalitis vector (*Culiseta melanura* (Coquillett)) were in forested wetlands. Eastern Equine Encephalitis can also infect humans. Schafer and Lunstrom (2001) compared mosquito species trapped in four forested wetlands in Sweden. There is a literature on managing forested wetlands, which, although not specifically for mosquito control, may be relevant to mosquito issues. An example is the review by Sun et al. (2001) on the hydrologic impacts of common forest uses, including drainage. Drainage has, particularly in the past, also been used as a source reduction technique for mosquito management. However the research also showed effects such as raised water tables following harvest in some situations, for

example in Cypress and other forested swamps and, incidentally, a habitat of *Culiseta melanura* (Hachiya et al. 2007). These are very relevent to the creation of, or increase in, mosquito larval habitats, although not surprisingly this is not mentioned in specifically forest-focussed research.

One method of managing freshwater mosquitoes, apart from larviciding, is source reduction and this may involve draining or filling the larval habitats as described by Shililu et al. (2007). Their work was on malaria vectors in a semiarid part of Africa (Eritrea) and, because of the severe health risk, local communities were enlisted to monitor and fill or drain ephemeral wetlands, though this would destroy wetland function. In that case the human health risk was a paramount consideration. Gu et al. (2006) showed theoretically that source reduction, by reducing oviposition sites, could lead to a longer mosquito life cycle (mosquitoes have to search for longer to find suitable sites to lay eggs) and that this could have important implications for reducing disease incidence (in that case malaria).

Constructed wetlands are a specific category of freshwater wetlands and these have generated a great deal of interest as discussed below.

Constructed wetlands

Constructed wetlands are increasingly being used for water treatment (stormwater, secondary treated effluent) and also contribute wetland values to the environment. Schafer et al. (2004) assessed biodiversity in natural and constructed wetlands in temperate Sweden. They found mosquito abundance and species richness higher in the natural wetlands than in the constructed ones, although adult nuisance mosquitoes were associated with all the wetlands. However constructed wetlands may also create or contribute to a mosquito problem. Russell (1999) cautioned that they provided mosquito habitats, and potential for recurrence of diseases that had been eliminated in Australia (e.g., malaria, filariasis). There has been a body of research conducted since then showing that mosquito larvae may be found in the wetlands. For example, Gingrich et al. (2006) found a range of species, including vectors of WNV, in constructed wetlands in Delaware USA. Other researches have focused on management strategies to minimise larval habitats by, for example, vegetation management (Thullen et al. 2002; Jiannino and Walton 2004; Walton and Jiannino 2005). Conversely, flooding felled and dried vegetation, especially *Typha* spp., may increase mosquito abundance (Walton and Jiannino 2005). Marsh design (Walton and Workman 1998; Diemont 2006) or activities such as periodic draining (Mayhew et al. 2004; Dale et al. 2007) indicate that design and maintenance are important for managing mosquito larvae to inhibit adult emergence.

Information gaps

In the area of impacts of mosquito management activites there are knowledge gaps that include: the role of mosquitoes in wetland ecology; long-term impacts on non-target organisms of larvicides; long-term impacts of habitat management on wetlands and, for forested wetlands, the need to identify the nature of larval habitats, suggest management options and evaluate impacts.

Restoration

There is a growing literature on restoring damaged wetlands. This is a complex problem and needs an integrated interdisciplinary approach such as in Wassen and Grootjans (1996), who described a key ecohydrology approach in the Netherlands. Keddy (1999), recognising that restoration tended to be somewhat ad hoc and based on individual cases, proposed a scheme to unify the process, using assembly rules and indicators of ecosystem integrity, applicable across the range of wetlands from intertidal to fresh. A recent review by Elliott et al. (2007) for estuaries, coastal and marine systems that have been damaged by human land uses, highlighted topics that are poorly understood, such as the ecological goods and services provided by wetlands and they proposed a clarified nomenclature. Zedler and Nelson (2001) wrote a handbook for an adaptive management approach to restoring tidal wetlands (mainly salt marshes), that detailed the practical work involved, for a wide range of physio-chemical and biological variables. Although not necessarily referring to mosquitoes the concepts noted above are useful guides for restoration.

Restoring wetlands that have been affected by mosquito control activities or other disturbance,

usually related to modifications such as ditching or impounding, is often in intertidal wetland areas and usually involves restoring tidal circulation. An early approach is exemplified by Broome et al. (1988). Although it does not specifically refer to mosquito-related disturbance, it does discuss water management and that could include mosquito management activities. However, Broome et al. do not consider the interruption to marsh evolution that may have resulted from disturbance over a lengthy period. This is addressed, for impounded marshes in Florida (USA), by Parkinson et al. (2006, cited above).

Restoration may also specifically include mosquito management. Much of the research on this topic has been conducted in USA. For ditched marshes restoration usually re-introduces tidal flushing by the OMWM approach introduced in the 1960s in New Jersey (USA) and mentioned above (Ferrigno and Jobbins 1968). Tidal flushing has also been restored, or partly restored, to impounded marshes in many areas. A temperate example is in Connecticut (USA), where Swamy et al. (2002) reported a long-term study and noted that recovery may take decades. Recovery may be more rapid in tropical and subtropical areas and research on RIM has been conducted on Florida (USA) marshes that were impounded in the 1950s and 1960s. The method takes into account the need for mosquito management especially during the summer and the need to conserve wetland values. It thus restores tidal access during autumn and winter, and floods the marshes in spring and summer, using floodgates and pumps if needed (Carlson and Carroll 1983; O'Bryan et al. 1990; Brockmeyer et al. 1997). Lewis and Gilmore (2007) provided a very useful review of mangrove forest restoration in Florida east coast wetlands that had been impounded for mosquito control. They suggested strategies for restoration but did not discuss the potential to also restore the mosquito populations that led to impoundmnet in the first place.

Restoring wetland function and benefitting wildlife may also have the unintended effect of creating mosquito habitat, though this may be mitigated by vegetation management (removal) (Lawler et al. 2007). An example from Australia that involved restoring tidal flushing to a wetland showed not only changes to the vegetation with colonisation by salt marsh and mangrove species (intended) but also increased oviposition by disease vector mosquitoes (unintended) (Turner and Streever 1999).

Information gaps

There is a need for a better understanding of how to restore wetland function without also creating or enhancing mosquito habitat. This requires interdisciplinary approaches, as integrating research from several areas is relevant to the complex issue of mosquitoes and wetlands. Because ecosystem time frames may be long ones it will require long-term research to assess the effectiveness of restoration as well as monitoring to check for developing mosquito larval habitats.

Impacts of change

While climate change is currently a focus of interest, there are also considerable direct and indirect impacts of human land use decisions on vector-borne disease and mosquitoes and some of these may be indirectly related to climate change. Patz et al. (2004) reviewed the effects of land use changes on a variety of mosquito-borne diseases. They also referred to the Millennium Ecosystem Assessment (noted by Pongsiri and Roman (2007), see below).

Specific land use changes such as deforestation can lead to environmental change conducive to mosquito success. Walsh et al. (1993) considered the effects of deforestation on a range of diseases. Previously cited, Yasuoka and Levins (2007) did the same more recently for malaria in Africa and Asia and Vittor et al. (2006) found large increases in anopheline mosquito biting rates in an area that had been deforested in the Peruvian Amazon. Land use change can have unexpected impacts on mosquito populations. Thus Lindsay et al. (2007), reviewing inland Western Australia, reported an increase in dryland salinity, possibly related to clearing for agriculture, that appeared to have led to the incursion of the salt marsh mosquito (*Aedes camptorhynchus* (Thomson)), a vector of Ross River virus. Derraik and Slaney (2007) reviewed the literature on anthropogenic change (referring also to climate change) and the potential effect on mosquitoes and mosquito-borne disease in New Zealand. Climate change is explored in the next section.

Climate change

In the last quarter of the twentieth century climate change was not widely accepted as a fact. With the fourth report of the IPCC (2007) this has changed and climate change and its health impacts are now receiving much attention. The literature that refers specifically to the effect of climate change on mosquito-borne disease goes back to the 1980s when the scenario was uncertain. One of the earliest publications was that of Liehne (1988) who considered the effects in Australia. On the simple expectation, as it then was, that rainfall and temperature would increase, Liehne predicted that malaria risk would increase in the tropical areas and the breeding season of *Culex annulirostris* would extend further south and that this could lead to an increased incidence of polyarthritis diseases such as Ross River virus. He also predicted an increase in the range and frequency of Australian encephalitis disease.

As interest in and concern about climate change and health has increased there has been an increasing number of papers on the topic since the mid 1990s (Fig. 2).

In a relatively early paper Patz and Balbus (1996) foresaw the need to model climate change scenarios and advocated the use of RS and GIS to assess risk related to climate change. Mellor and Leake (2000) reviewed the climatic factors important for vector-borne disease in order to predict climate change effects. Githeko et al. (2000) reviewed the implications of climate change on a regional basis. In summary they expected that the greatest impacts would be felt at the extremes of the temperature ranges at which transmission occurs, via effects on incubation rates and mosquito survival. For specific areas they expected particular outcomes and these are summarised in Table 3 below. They concluded for all areas that the effects of climate change are likely to be variable.

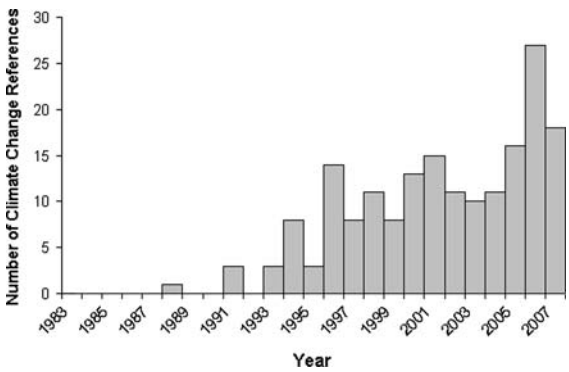


Fig. 2 Climate change references by year

Reiter (2001) took a more conservative approach in his review of the history of the major mosquito-borne diseases (malaria, yellow fever and dengue). He noted that " the natural history of mosquito-borne diseases is complex, and the interplay of climate, ecology, vector biology and many other factors defies simplistic analysis." (p. 158). Githeko et al. (2000) and Reiter (2001) both noted that the effects of climate-induced disease risk may be offset by high standards of living. Hunter (2003) reviewed the potential impacts of increased rainfall and higher temperatures on vector-borne disease (and water borne disease). Kuhn et al. (2007) reviewed the use of climate to predict infectious diseases including mosquito-borne disease, in the light of climate variability and change. That was published prior to the most recent IPCC predictions, but is still relevant to developing early warning systems that would assist disease management and have potential wetland consequences.

Recent papers by Watson et al. (2005), Patz et al. (2005) and Sunyer and Grimalt (2006) discussed the likely impacts of climate change, on vector-borne disease. Watson et al. (2005), in their critical review, noted the need for very broad environmental monitoring and that this should also include responses of biological systems to climate change. A recent paper by McMichael et al. (2006) reviewed the literature on climate change and health at a global level and included a figure that summarised the pathways whereby climate change may affect human health, including the impact on natural systems (and this would include wetlands).

The use of RS, GIS and other climate change modelling approaches is growing. Some predictive modelling has been done on disease risk, based on vector ecology and likely climate changes. An example is the Hotspots models of de Wet et al. (2005) that assessed the risk of Australian disease vector mosquitoes arriving in or dispersing in New Zealand. Zou et al. (2007) used a relatively simple GIS to estimate the risk of WNV; Eisele et al. (2003) focused on malaria in Africa, as did Ceccato et al. (2005). Thomson et al. (1996, 2005) showed that satellite RS, even at low resolution, could assist in predicting

Table 3 Potential impacts of climate change at a regional level (based on Githeko et al. 2006)

Place	Potential effect
Africa	The response is likely to be variable as the effects of climate change are not evenly spread, nor are the disease implications simple. Research is cited showing that increases in temperature and rainfall have been associated with increased malaria in Kenya, but the same climate effects were accompanied by a decline in malaria in the United Republic of Tanzania.
Europe	Malaria was once common and it could return with climate change and the expanded range of vector habitats. However, the risk is low in the wealthier nations (because of social and health services) though it may be high in impoverished areas.
South America	Malaria and dengue are the major disease risks, though there are cases of yellow fever and encephalitis. The strong effect of El Nino in the equatorial areas is likely to increase the incidence of malaria and dengue and the effects may be exacerbated by any increase in poverty.
North America	Health risks will be affected by the public health and vector control systems that are in place. In southern areas (e.g., Mexico) the infrastructure is less developed and hence there may be increases in diseases such as malaria. They report research that shows that diseases such as St Louis encephalitis may move north as warming occurs.
Asia, Australia, Western Pacific	Changes to the El Nino effect may lead to increased diseases such as malaria in, for example, India. Australia and New Zealand are at risk of increases in the existing arbovirus diseases such as Ross River and Barmah Forest viruses and Murray Valley encephalitis.

malaria outbreaks in Africa, using the Normalised Difference Vegetation Index (NDVI) as an indicator of antecedent rainfall in larval habitats and a precursor to adult mosquito emergence. Other modeling that is relevant includes research on the relationship between mosquitoes and other variables that are likely to be affected by climate change. An example is research into Ross River virus in Queensland, Australia whereby relationships are shown between the disease and climate (especially rainfall) and tides (sea level) (Kelly-Hope et al. 2004; Tong et al. 2005), climatic variability (Tong and Hu 2002; Tong et al. 2004) or all of these (Naish et al. 2006).

Crane et al. (2005), in the context of chemical control of insects, developed a risk model for climate change and its impacts on insect-borne disease. While it focused on using chemicals to control insects it did so with the objective of protecting aquatic life and hence the approach is relevant to wetland conservation. As with many others, Crane et al. noted the lack of sufficient information about environmental impacts of climate change and the systems that may be affected.

Information gaps

There is information on the likely effects of climate change on mosquitoes and on the diseases they transmit but little that also addresses whole system impacts and management (and by implication wetland issues). As wetlands are likely to be directly and indirectly impacted by climate change there is a need to integrate that information with information on mosquito management.

Balancing the environment and human health issues: adaptive approaches

The issue of human health and biodiversity has been addressed by Pongsiri and Roman (2007), including specific reference to mosquito-borne disease. They reported a Millennium Ecosystem Assessment that showed the need for understanding biodiversity and its relationship with health. Can we manage both for mosquitoes and also sustain the environment? This question was addressed in Dale (1993), with the view that minimal modification of suitable habitats (salt marshes) is one acceptable solution. Others have also addressed the issue, for example O'Bryan et al. (1990) and Carlson et al. (1999). There are policy documents that outline management from the environmental perspective, for example the recent draft policy for mosquito management in wildlife refuges in USA that acknowledged both the environmental values and the health risks (Department of the Interior 2007).

Adaptive management of wetlands is the subject of research that may be relevant to the interaction between mosquito management and wetland management. McWilliams et al. (2007) used an adaptive

approach to impact assessment but also stressed the need to include on-going monitoring, as simple manipulations may have complex results. Olsson et al. (2004) recounted an adaptive management process for managing wetlands in southern Sweden that involved all stakeholders. This is a model that could be used to reconcile what are often seen as conflicting priorities between mosquito control and habitat management. It is similar to the approach of Carlson (1986) describing the Florida Technical Subcommittee on Managed Salt Marshes that was established in 1983 (renamed in 1985) and that resulted in collaboration not confrontation. Other references to adaptive management in a wetland context include La Peyre et al. (2001) and Dale et al. (2006).

There is a risk that wetland management may inadvertently increase mosquito populations. In constructed wetlands the potential is acknowledged and can be avoided by design and maintenance principles. However management in other areas such as in wetland restoration projects may have the unexpected effect of creating conditions suitable for mosquitoes. For example, managing water levels may favour aedine mosquitoes. An example of water level management without apparent reference to mosquitoes is reported in Palliason et al. (2006) who discuss the effect of water level management to control water lilies in west France and its effect on their species of interest: populations of whiskered tern (*Chlidonias hybridus*). For other examples of conservation focused wetland management research whose results may have mosquito implications, though these are not the subject of the research, see Self (2005) and Connor and Gabor (2006).

Information gaps

In the area of balancing health and wetland interests there is not much that covers both. There has been, and is, a wealth of literature on wetland management that recognises the range of issues that need to be addressed and approaches that can be used (see for example Gosselink et al. 1990; Doust and Doust 1995; Zacharias et al. 2005) including an emphasis on the importance of hydrology, as reviewed in Gilvear and Bradley (2000). Information about mosquitoes and wetlands needs to be integrated into management to help achieve a balance between what may be conflicting interests.

Conclusion

To summarise, wetlands have high intrinsic value and also provide habitats for the immature stages of mosquitoes. Mosquitoes are perceived to be a nuisance and demonstrably transmit diseases. Management of the insect often focuses on the habitats of the immature stages and so wetlands are impacted by mosquito management activities. Information on local mosquito habitats may be held by mosquito managers but little is published in the refereed scientific literature. Because wetland values are important, mosquito management activities that impact them need to be integrated into overall management plans. There needs to be communication and cooperation between mosquito management and wetland management in order to balance the interests of each and to sustain both wetland and human health, especially in the context of changing land use and climatic environments. As the interaction between wetlands, mosquitoes and mosquito-borne disease is complex there is also a need for interdisciplinary research to provide information that is useful to management.

Finally, a limitation of this review is that it is largely restricted to peer reviewed publications in the English language and thus may have missed important information published in other languages. This is an aspect that could perhaps be explored in the future by an international, multilingual team.

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COMPARATIVE EFFICACY OF INSECT REPELLENTS AGAINST MOSQUITO BITES

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ABSTRACT

Background The worldwide threat of arthropod-transmitted diseases, with their associated morbidity and mortality, underscores the need for effective insect repellents. Multiple chemical, botanical, and "alternative" repellent products are marketed to consumers. We sought to determine which products available in the United States provide reliable and prolonged complete protection from mosquito bites.

Methods We conducted studies involving 15 volunteers to test the relative efficacy of seven botanical insect repellents; four products containing *N,N*-diethyl-*m*-toluamide, now called *N,N*-diethyl-3-methylbenzamide (DEET); a repellent containing IR3535 (ethyl butylacetylaminopropionate); three repellent-impregnated wristbands; and a moisturizer that is commonly claimed to have repellent effects. These products were tested in a controlled laboratory environment in which the species of the mosquitoes, their age, their degree of hunger, the humidity, the temperature, and the light–dark cycle were all kept constant.

Results DEET-based products provided complete protection for the longest duration. Higher concentrations of DEET provided longer-lasting protection. A formulation containing 23.8 percent DEET had a mean complete-protection time of 301.5 minutes. A soybean-oil–based repellent protected against mosquito bites for an average of 94.6 minutes. The IR3535-based repellent protected for an average of 22.9 minutes. All other botanical repellents we tested provided protection for a mean duration of less than 20 minutes. Repellent-impregnated wristbands offered no protection.

Conclusions Currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in environments where mosquito-borne diseases are a substantial threat. (N Engl J Med 2002;347:13-8.)

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INSECT-TRANSMITTED disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million persons annually.[1] Malaria kills 3 million persons each year, including 1 child every 30 seconds.[2,3] Although insect-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to their risks. In the United States, arboviruses transmitted by mosquitoes continue to cause sporadic outbreaks of eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, and La Crosse encephalitis.[4,5] In the fall of 1999, West Nile virus, transmitted by mosquitoes, was detected for the first time in the Western Hemisphere. In the New York City area, 62 persons infected with West Nile virus were hospitalized, and 7 persons died.[6-8] The Centers for Disease Control and Prevention estimates that more than 2000 persons were infected with West Nile virus in the year 2000.[9] The virus has now been detected in 27 states, and it is anticipated that it will continue to spread unabated across the United States during the next few years.[9,10]

Protection from arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing, and using insect repellent.[11,12] In many circumstances, applying repellent to the skin may be the only feasible way to protect against insect bites. Given that a single bite from an infected arthropod can result in transmission of disease, it is important to know which repellent products can be relied on to provide predictable and prolonged protection from insect bites. Commercially available insect repellents can be divided into two categories — synthetic chemicals and plant-derived essential oils. The best-known chemical insect repellent is *N,N*-diethyl-*m*-toluamide, now called *N,N*-diethyl-3-methylbenzamide (DEET). Many consumers, reluctant to apply DEET to their skin, deliberately seek out other repellent products. We compared the efficacy of readily available alternatives to DEET-based repellents in a controlled laboratory environment.

METHODS

Product Selection

In January 2001, we purchased a total of 16 products for testing, choosing repellents with national, rather than local, distribution (Table 1). Seven widely available botanical repellents were included in the study. Multiple concentrations and formulations of DEET are readily available. We chose and tested three DEET-based repellents (ranging from 4.75 to 23.8 percent DEET) that we believe represented the range of commonly purchased repellents in the United States. We also tested a controlled-release 20 percent DEET formulation to determine whether it had a longer duration of action. The only synthetic repellent containing IR3535 (ethyl butylacetylaminopropionate) that is available in the United States and three

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wristbands impregnated with either DEET or citronella were also tested. Finally, we tested the efficacy of a proprietary moisturizer that is commonly believed to have repellent effects.

Testing Methods

The duration of protection provided by each product was tested by means of arm-in-cage studies, in which volunteers insert their repellent-treated arms into a cage with a fixed number of unfed mosquitoes, and the elapsed time to the first bite is recorded. Testing of repellents is usually conducted either in a laboratory or at outdoor field sites.[14] Conducting such studies indoors makes it possible to reduce potential confounding variables, such as wind speed, temperature, humidity, density of the mosquito population, the level of the mosquitoes' hunger, and the species of the mosquitoes, that can make it difficult to interpret comparisons among products made in outdoor-field trials. We conducted our tests with a low density of mosquitoes per cage rather than a high density (some studies use more than 250 mosquitoes per cage) because the low-density environment more accurately reflects the typical biting pressures that are encountered during most outdoor activities.

For each test, 10 disease-free, laboratory-reared *Aedes aegypti* female mosquitoes that were between 7 and 24 days old were placed into separate laboratory cages measuring 30 cm by 22 cm by 22 cm. A batch of 10 mosquitoes that had not been exposed to the repellent being tested was used for each arm insertion. Mosquitoes were provided with a constant supply of 5 percent sucrose solution. Cages were placed in a walk-in incubator measuring 2.2 m by 2.2 m by 2.2 m, in which the temperature was maintained at 24 to 32°C, the relative humidity at 60 to 70 percent, and the light–dark cycle at 12 hours of light followed by 12 hours of darkness.

Fifteen volunteers (5 men and 10 women) were recruited from the staff of the Medical Entomology Laboratory at the University of Florida. The study was reviewed and approved by the institutional review board of the University of Florida, and subjects gave written informed consent before participating.

As repellents were purchased, they were labeled sequentially from 1 to 16. A random-number generator was then used to determine the order in which the products would be tested on each subject. A total of 720 individual tests were conducted, with each repellent being tested three times on each subject. Most subjects only completed one test per day. The average time to completion of all three tests was 10.2 days. In the case of repellents that were identified as very short-acting in the initial test, subjects were permitted to conduct all three tests of the repellent in a single day, washing the skin with an unscented soap before each application of the repellent. Subjects did not test more than one repellent product on a single day. No information on the likely duration of action of each repellent was provided to subjects before they began their tests.

Before each test, the readiness of the mosquitoes to bite was confirmed by having subjects insert their untreated forearm into the test cage. Once subjects observed five mosquito landings on the untreated arm, they removed their arm from the cage and applied the repellent being tested from the elbow to the fingertips, following the instructions on the product's label. After the application of the repellent, subjects were instructed not to rub, touch, or wet the treated arm. Repellent-impregnated wristbands were worn on the wrist of the arm being inserted into the cage. Subjects were provided with a standardized log sheet to ensure accurate documentation of the duration of exposure and the time of the first bite. The elapsed time to the first bite was then calculated and recorded as the "complete-protection time" for that subject in that particular test.

Subjects were asked to follow the testing protocol shown in Figure 1. Subjects conducted their first test of each repellent by inserting the treated arm into a test cage for one full minute every five minutes. If they were not bitten within 20 minutes, then the arm was reinserted for 1 full minute every 15 minutes, until the first bite occurred. On the basis of this initial complete-protection time, the subject's next two tests of that particular repellent were conducted as follows: if the repellent had initially worked for less than 20 minutes, the subject placed his or her arm in the cage for 1 minute every 5 minutes; if the repellent had initially worked for 20 minutes to 4 hours, the subject placed his or her arm in the cage for 1 minute every 15 minutes; and if the repellent had initially worked for more than 4 hours, the subject placed his or her arm in the cage for 1 minute every hour (up to 4 hours). If a repellent was still working after 4 hours, then the subject continued to place his or her arm in the cage every 15 minutes thereafter, until the first bite occurred. If at any point during testing, subjects noted mosquitoes landing but not biting (a behavior that typically occurs when the efficacy of a repellent begins to wane), then the intervals between insertions were decreased to five minutes.

Discretionary funds from the State of Florida were used to support this study. We received no financial support from industry, including the manufacturers whose products were tested in the study. Data analysis was performed within the Florida Medical Entomology Laboratory at the University of Florida, without input from any outside sources.

Statistical Analysis

Two-way analysis of variance (involving two factors, subject and repellent) followed by Tukey's tests[13] was used to compare the mean complete-protection time for the 16 tested repellents. All P values are two-sided; a P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Of the products tested, those containing DEET provided the longest-lasting protection (Table 1). The complete-protection times of DEET-based repellents correlated positively with the concentration of DEET in the repellent. The formulation containing 4.75 percent DEET provided an average of 88.4 minutes of complete protection; the formulation containing 23.8 percent DEET protected for an average of 301.5 minutes. There was a statistically significant difference in complete-protection time between each DEET-based repellent and the product with the next higher concentration of DEET ($P<0.001$ for all comparisons). The controlled-release formulation we tested did not prolong the duration of action of DEET. The alcohol-based product containing 23.8 percent DEET protected significantly longer than the controlled-release formulation containing 20 percent DEET ($P<0.001$).

No non-DEET repellent fully evaluated in this study was able to provide protection that lasted more than 1.5 hours. Only the soybean-oil–based repellent was able to provide protection for a period similar to that of the lowest-concentration DEET product we tested (94.6 and 88.4 minutes, respectively).

The IR3535-based repellent protected against mosquito bites for an average of 22.9 minutes. The citronella-based repellents we tested protected for 20 minutes or less. There was no significant difference in protection time between the slow-release formulation containing 12 percent citronella and the formulation

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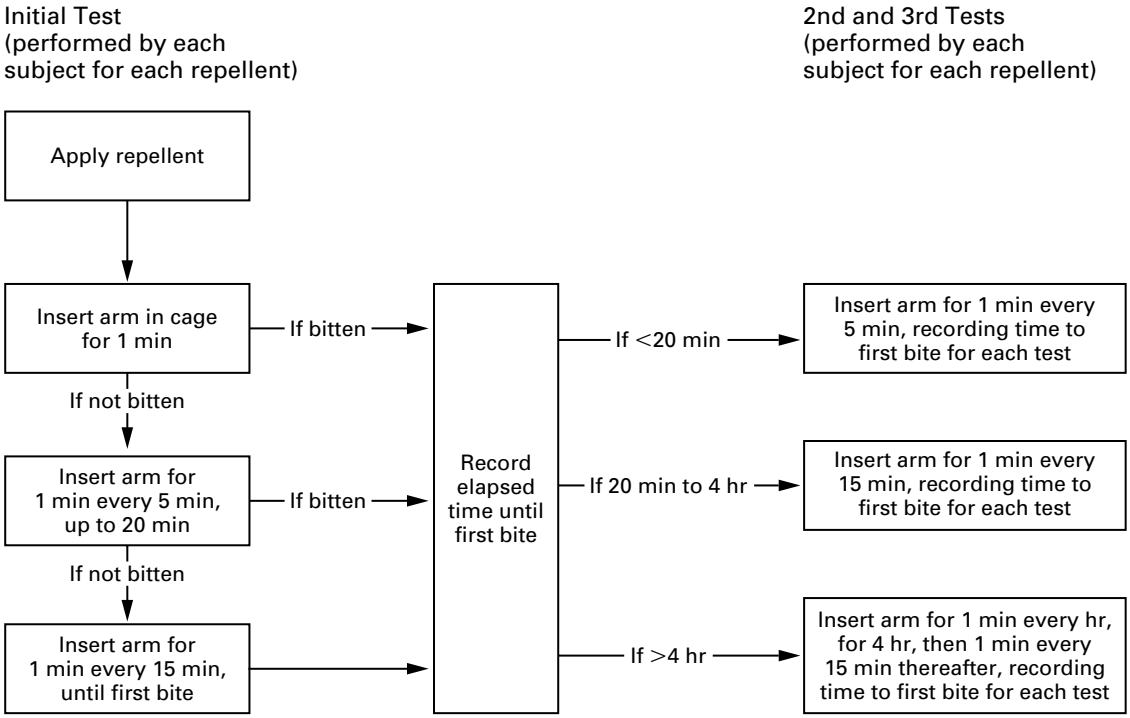

Figure 1. Study Design.

If at any time during testing, mosquitoes were seen to land on the skin but not bite (a sign of imminent failure of the repellent), then the interval between insertions was decreased to five minutes until the first bite was confirmed.

containing 5 percent citronella (P=0.07) or the two formulations containing 10 percent citronella (P=0.16 and P=0.80). The repellent containing only 0.05 percent citronella provided less protection than the Skin-So-Soft mineral-oil–based moisturizer (Avon) (P<0.001). Repellent-impregnated wristbands, containing either 9.5 percent DEET or 25 percent citronella (by weight), protected the wearer for only 12 to 18 seconds, on average.

In arm-in-cage studies, testing must be conducted with insertions at limited intervals, with a new batch of mosquitoes for each test, because continuous exposure may cause mosquitoes to fatigue or may induce prolonged blockage of their antennal chemoreceptors, both of which will prevent further biting. Conducting tests of a repellent in which the arm is inserted into the cage at fixed intervals, however, has some obvious limitations. A repellent might stop working between the removal of the arm and the subsequent insertion, but the failure would not be detected until the next scheduled insertion, causing an inflated measure of the duration of protection provided by that repellent. In our study, the greatest risk of overestimation of complete-protection times would affect the repellents that were tested with once-hourly insertions into the cage. According to our protocol, however, hourly insertions were only used by subjects who found that a repellent initially protected them for more than four hours. Only the two highest-concentration DEET-based repellents in our study (20 percent and 23.8 percent DEET) qualified for once-hourly insertions by some of the subjects, and the range of protection these repellents afforded (180 to 360 minutes) is consistent with previously published reports of the efficacy of DEET.[15,16] Any rounding errors resulting from the intervals between insertions into the cage would also tend to overestimate the efficacy of the other repellents we tested, and 11 of the 12 non-DEET products still had mean complete-protection times of less than 23 minutes.

After the original studies for this article were completed, a new botanical repellent was introduced in the United States. The repellent contains oil of eucalyptus and is marketed under two names: Repel Lemon Eucalyptus Insect Repellent (WPC Brands)

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TABLE 1. PROTECTION TIMES OF INSECT REPELLENTS.*

PRODUCT	ACTIVE INGREDIENT AND CONCENTRATION	COMPLETE-PROTECTION TIME		CATEGORY OF PROTECTION†
		MEAN	RANGE	
		min		
OFF! Deep Woods (SC Johnson)	DEET, 23.8%	301.5±37.6	200–360	A
Sawyer Controlled Release (Sawyer)	DEET, 20%	234.4±31.8	180–325	B
OFF! Skintastic (SC Johnson)	DEET, 6.65%	112.4±20.3	90–170	C
Bite Blocker for Kids (HOMS)	Soybean oil, 2%	94.6±42.0	16–195	D
OFF! Skintastic for Kids (SC Johnson)	DEET, 4.75%	88.4±21.4	45–120	D
Skin-So-Soft Bug Guard Plus (Avon)	IR3535, 7.5%	22.9±11.2	10–60	E‡
Natrapel (Tender)	Citronella, 10%	19.7±10.6	7–60	E‡
Herbal Armor (microencapsulated) (All Terrain)	Citronella, 12%; peppermint oil, 2.5%; cedar oil, 2%; lemongrass oil, 1%; geranium oil, 0.05%	18.9±13.3	1–55	E§
Green Ban for People (Mulgum Hollow Farm)	Citronella, 10%; peppermint oil, 2%	14.0±11.3	1–45	E
Buzz Away (Quantum)	Citronella, 5%	13.5±7.5	5–30	E
Skin-So-Soft Bug Guard (Avon)	Citronella, 0.1%	10.3±7.9	1–30	E
Skin-So-Soft Bath Oil (Avon)	Uncertain¶	9.6±8.8	1–30	E
Skin-So-Soft Moisturizing Suncare (Avon)	Citronella, 0.05%	2.8±3.4	1–15	F
Gone Original Wristband (Solar Gloooow)	DEET, 9.5%	0.3±0.2	0.17–1.33	G
Repello Wristband (Repello Products)	DEET, 9.5%	0.2±0.08	0.17–0.63	H
Gone Plus Repelling Wristband (Solar Gloooow)	Citronella, 25%	0.2±0.09	0.17–0.48	H

*Plus–minus values are the means ±SD of the times to the first bite in the tests of all 15 subjects. DEET denotes *N,N*-diethyl-3-methylbenzamide (formerly known as *N,N*-diethyl-*m*-toluamide), HOMS Home Operations and Management Systems, and IR3535 ethyl butylacetylaminopropionate.

†The mean complete-protection time of each repellent was significantly different ($P<0.05$ by analysis of variance and Tukey's tests[13]) from those of all repellents in different categories of protection (A, B, C, D, E, F, G, and H).

‡The complete-protection time also differed significantly from those of Buzz Away, Skin-So-Soft Bug Guard, and Skin-So-Soft Bath Oil.

§The complete-protection time also differed significantly from those of Skin-So-Soft Bug Guard and Skin-So-Soft Bath Oil.

¶This product contains mineral oil, isopropyl palmitate, dicapryl adipate, fragrance, dioctyl sodium sulfosuccinate, butylated hydroxytoluene, and carrot oil.

and Fite Bite Plant-Based Insect Repellent (Travel Medicine). We evaluated this type of repellent using the same testing methods in six subjects (five men and one woman). In one subject, a localized cutaneous reaction developed after the first test, and the subject discontinued the study. All other subjects completed three tests each of the repellent. The repellent had a mean (±SD) complete-protection time of 120.1±44.8 minutes, with a range of 60 to 217 minutes.

DISCUSSION

Protection against arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing, and applying insect repellent.[11,12] The insect repellents that are currently available to consumers are either synthetic chemicals or are derived from plants. The most widely marketed chemical-based insect repellent is DEET, which has been used worldwide since 1957. DEET is a broad-spectrum repellent that is effective against many species of mosquitoes, biting flies, chiggers, fleas, and ticks.[17] The protection provided by DEET is proportional to the logarithm of the dose; higher concentrations of DEET provide longer-lasting protection, but the duration of action tends to plateau at a concentration of about 50 percent.[18] Most commercially available formulations now contain 40 percent DEET or less, and the higher concentrations are most appropriate to use under circumstances in which the biting pressures are intense, the risk of arthropod-transmitted disease is great, or environmental condi-

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tions promote the rapid loss of repellent from the surface of the skin.[19] In our study, a formulation containing 23.8 percent DEET provided an average of five hours of complete protection against *A. aegypti* bites after a single application. Depending on the formulation and concentration tested, DEET-based repellents have been shown in other studies to provide complete protection against arthropod bites for as long as 12 hours, even under harsh climatic conditions.[20,21]

The most recent addition to the synthetic insect repellents on the market in the United States is IR3535,[22] which is classified by the Environmental Protection Agency as a biopesticide because of its structural similarity to the amino acid alanine. This repellent has been used in Europe for more than 20 years and was approved for use in the United States in 1999. In our tests, this repellent fared poorly, yielding a mean complete-protection time that was one quarter that of the lowest-concentration DEET product we tested (22.9 vs. 88.4 minutes).

Skin-So-Soft Bath Oil, which consumers commonly claim has a repellent effect on insects, provided only a mean of 9.6 minutes of protection against aedes bites in our study. This extremely limited repellent effect has previously been documented in other studies.[15]

Thousands of plants have been tested as potential botanical sources of insect repellent.[23-25] Most plant-based insect repellents currently on the market contain essential oils from one or more of the following plants: citronella, cedar, eucalyptus, peppermint, lemongrass, geranium, and soybean. Of the products we tested, the soybean-oil–based repellent was able to protect from mosquito bites for about 1.5 hours. All other botanical repellents that we tested in our initial studies, regardless of their active ingredients and formulations, gave very short-lived protection, ranging from a mean of about 3 to 20 minutes. Preliminary studies suggest that the oil-of-eucalyptus products will confer longer-lasting protection than other available plant-based repellents.

Most alternatives to topically applied repellents have proved to be ineffective. No ingested compound, including garlic and thiamine (vitamin B_1), has been found to be capable of repelling biting arthropods.[26-28] Small, wearable devices that emit sounds that are purported to be abhorrent to biting mosquitoes have also been proved to be ineffective.[29] In our study, wristbands impregnated with either DEET or citronella similarly provided no protection from bites, consistent with the known inability of repellents to protect beyond 4 cm from the site of application.[30]

Multiple factors play a part in determining how effective any repellent will be; these factors include the species of the biting organisms and the density of organisms in the immediate surroundings; the user's age, sex, level of activity, and biochemical attractiveness to biting arthropods; and the ambient temperature, humidity, and wind speed.[31-34] As a result, a given repellent will not protect all users equally. Examination of the ranges of complete-protection times in Table 1 shows variation in the ability of each repellent to protect different subjects. Thus, these times should be taken not as absolute values but, rather, as an indication of the relative effectiveness of the tested repellent products.

Our study shows that only products containing DEET offer long-lasting protection after a single application. Certain plant-derived repellents may provide short-lived efficacy, which may be sufficient when arthropod bites are primarily a nuisance. Frequent reapplication of these repellents would partially compensate for their short duration of action. However, when one is traveling to an area with prevalent mosquito-borne disease that could be transmitted through a single bite, the use of non-DEET repellents would seem to be ill-advised. Given our findings, we cannot recommend the use of any currently available non-DEET repellent to provide complete protection from arthropod bites for any sustained outdoor activity.

Although this study shows that DEET-based products can be depended on for long-lasting repellent effect, they are not perfect repellents. DEET may be washed off by perspiration or rain, and its efficacy decreases dramatically with rising outdoor temperatures.[19,30,34] DEET is also a plasticizer, capable of dissolving watch crystals, the frames of glasses, and certain synthetic fabrics.

Despite the substantial attention paid by the lay press every year to the safety of DEET, this repellent has been subjected to more scientific and toxicologic scrutiny than any other repellent substance. The extensive accumulated toxicologic data on DEET have been reviewed elsewhere.[17,35-39] DEET has a remarkable safety profile after 40 years of use and nearly 8 billion human applications.[35] Fewer than 50 cases of serious toxic effects have been documented in the medical literature since 1960, and three quarters of them resolved without sequelae.[35,37] Many of these cases of toxic effects involved long-term, heavy, frequent, or whole-body application of DEET. No correlation has been found between the concentration of DEET used and the risk of toxic effects. As part of the Reregistration Eligibility Decision on DEET, released in 1998, the Environmental Protection Agency reviewed the accumulated data on the toxicity of DEET and concluded that "normal use of DEET does not present a health concern to the general U.S. population."[40] When applied with common sense, DEET-based repellents can be expected to provide a safe as well as a long-lasting repellent effect. Until a better repellent becomes available, DEET-based re-

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pellents remain the gold standard of protection under circumstances in which it is crucial to be protected against arthropod bites that might transmit disease.

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DENSITY AND DIVERSITY OF NONTARGET INSECTS KILLED BY SUBURBAN ELECTRIC INSECT TRAPS[1]

Timothy B. Frick, Douglas W. Tallamy[2]

ABSTRACT: Our survey of insects electrocuted during routine use of electric insect traps revealed only 31 biting flies, a minute proportion (0.22%) of the 13,789 total insects counted. In contrast, species from 12 orders and more than 104 nontarget insect families, including 1,868 predators and parasites (13.5%) and 6,670 nonbiting aquatic insects (48.4%) were destroyed. The heavy toll on nontarget insects and the near absence of biting flies in catches suggests that electric insect traps are worthless for biting fly reduction — and probably are counterproductive — to homeowners and other consumers.

Electric insect traps (*e.g.*, Zapper™, Bugwacker™ and Bug Blaster™; hereafter, "zappers") use ultraviolet light to lure flying insects toward an electrified metal grid, where they are destroyed by the thousands on warm summer nights. Homeowners buy traps to rid their surroundings of annoying biting flies, and continuous snaps, crackles, and pops emanating from an active zapper seem to confirm their effectiveness. Traps are commonly used near aquatic habitats, waterfront areas, toll booths, campgrounds, industrial parks, restaurants, swimming pools, and suburban backyards. In suburban yards, traps are often run throughout the summer months, some only during the evening hours and some continually.

Although the target insects are primarily mosquitoes (Culicidae) and no-see-ums (Ceratopogonidae) that seek blood meals at the expense of homeowners, several factors make electric traps ineffective in reducing local mosquito populations (Surgeoner & Helson 1977, Nasci *et al.* 1983). Ultraviolet lamps that emit considerable amounts of visible light (as do the lamps sold in commercial electric traps) are less attractive to mosquitoes than lamps emitting only ultraviolet wavelengths (Ikeuchi 1967). Furthermore, many species of mosquitoes are not attracted to light traps at all (Pippin 1965, Miller *et al.* 1969) and those species that are are often not trapped in numbers proportionate to their population sizes (Bradley 1943, Huffaker & Back 1943, Fox 1958). But perhaps the most important reasons electric insect traps fail to reduce mosquito problems are that 1) carbon dioxide exhaled by homeowners is far more attractive to mosquitoes than are light traps (Headlee 1941, Huffaker & Back 1943, Nascit *et al.* 1983), and 2) mosquitoes that do move toward traps are rarely killed by electrocution devices (Service 1993).

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Electric insect traps are, however, effective at killing large numbers of nontarget insects. Nasci *et al.* (1983) found that the average zapper in South Bend, Indiana killed more than 3000 insects per day, 96.7% of which were not female mosquitoes. Little beyond ordinal totals is known about the diversity and seasonal distribution of nontarget insects killed by zappers. As an initial step toward understanding the ecological consequences of indiscriminant removal by zappers of nontarget predators, parasitoids, and prey species from aquatic and terrestrial ecosystems, we quantified at the family level the numbers and kinds of insects killed over a season by homeowners' zappers in a suburban setting.

MATERIALS AND METHODS

We asked six homeowners with active bug zappers in suburban Newark, Delaware to participate in a summer-long study in 1994. All houses were within 3 km of a body of water. The house closest to water was about 65 meters from a large stream containing many stagnant eddies. Another house abutted a wooded area and was less than 1 km from a creek. The third house was about 1.5 km from the same creek but farther upstream. The fourth was in a wooded cul-de-sac through which ran a different creek; several permanent pools lay within 200 meters. The fifth house was situated in a residential development containing a stream and scattered wooded areas; a small pond about 30 meters long and 15 meters wide was less than a kilometer away. A small stream about 3 km distant was the nearest body of permanent water to the sixth house. Temporary pools, tree holes and water-filled containers were scattered throughout the study area. Thus, all traps were well within flight range of culicid and ceratopogonid breeding sites.

From June 20 to July 9, 1994, homeowners were asked to run the traps one night per week for at least two hours. Beginning on July 10, participants were asked to run their zappers one night per week every other week for the nine weeks ending August 27. A device constructed from a plastic dish 32 centimeters in diameter was suspended beneath each trap to collect electrocuted insects. Each morning after the traps were run, we collected the samples from the six sites and stored them in a freezer until they could be counted and identified to family (except for Ephemeroptera, Psocoptera, Thysanoptera, and Trichoptera, which were identified only to order, and several families of moths, which were grouped as "Microlepidoptera").

RESULTS

We collected 31 samples from the traps over our ten-week study period in the summer of 1994. Nearly all electrocuted specimens, including the tiniest

Cecidomyiidae, were well-preserved and easily identified. Twelve orders and more than 104 families were present in these samples and ranged in abundance from a single individual (several families) to more than 4,600 individuals (Chironomidae; Table 1). Of the 13,789 insects killed by electric zappers in our study, only 31 individuals (0.22%) were biting flies (female Culicidae, Simuliidae, and Ceratopogonidae). In contrast, insect predators, parasitoids, and nonbiting aquatic insects were abundant (Table 1). Present in our counts were representatives of 27 families of predators and nine families of parasitoids, totaling 1,868 individuals (13.5%). Carabid beetles, staphylinid beetles, cicadellid leafhoppers, microlepidoptera, and braconid parasitoids were particularly common victims. Large numbers of aquatic insects, such as caddisflies (Trichoptera) and midges (Chironomidae), were also destroyed; species from these families represented nearly half (48.4%) of sample totals.

Average numbers of insects per trap declined sharply over the season (Fig. 1), ranging from a mean of 1,304 insects per trap on June 20 to just 106 insects per trap on August 27. This probably reflects seasonal declines in the popula-

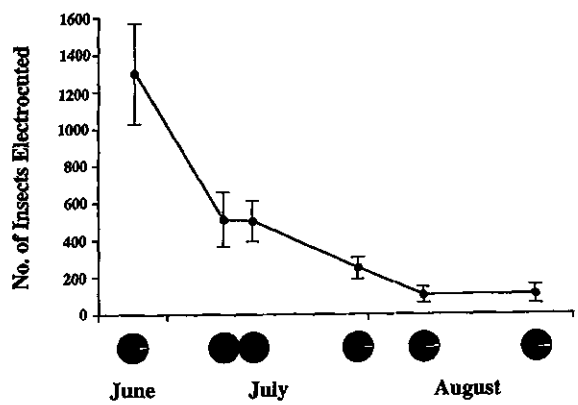

Fig. 1. Seasonal pattern of insects killed at six electric insect traps in Newark, DE on six dates from June 20 to August 27, 1994. Statistical interval = Standard Error. Pie charts depict the percentage of the total catch consisting of nontarget insects (black portion) and biting flies (white portion) on each trapping date

tions of species attracted to these traps. Although biting insects generally increased in proportion as the season progressed (from 0.26% of the total catch on June 20 to 1.88% on August 20), they still comprised a minuscule portion of the total sample.

DISCUSSION

These data are straightforward: many thousands of nontarget insects representing a rich taxonomic diversity were destroyed by these traps. Only a tiny fraction of trap victims were biting flies, the primary targets of electric zappers. Since we did not independently measure mosquito populations in our study sites we cannot definitively conclude that the zappers used in our study were ineffective mosquito killers. However, three types of circumstantial evidence suggest that this was indeed the case. First, it is highly unlikely that our lowland, wooded sites which were rich in aquatic breeding habitats, produced so few adult mosquitoes in the course of 9 weeks that 18 electrocuted females would represent adequate control of these flies. Second, the preponderance of aquatic insects in the samples suggests that our study traps were well within the flight range of biting flies that breed in water (culicids, ceratopogonids). Finally, our results are similar to those of Nasci *et al.* (1983) in which an independent measure of culicid populations confirmed the inability of zappers to attract mosquitoes that are present in suburban settings.

As we better understand the critical role insects play in the cohesion of most non-marine ecosystems, the sale and use of electric insect traps that so completely miss their advertised mark becomes increasingly irresponsible. It is insects and other invertebrates, not vertebrates, that are the "glue" of ecosystems; their elimination would inevitably lead to the rapid demise of those ecosystems and their members, including *Homo sapiens* (Wilson 1987). Even if targeted biting flies were effectively controlled by electric zappers, the resulting destruction of thousands of parasitoids, predators, aquatic insects, and other members of the nocturnally active fauna would be difficult to justify.

Although we recognize its speculative shortcomings, a simple calculation underscores the degree to which electric zappers may affect nontarget insect populations. The seasonal mean catch per night (of at least 2 hr of trap time) as quantified by our study totaled 445 insects per trap. Approximately one million zappers are sold in the U.S. each year (Philadelphia Inquirer, 26 June 1995 p. 63). Electrocution devices are quite durable; the homeowners in our study had been operating their units for an average of 7 yrs prior to 1994. If, in any given year, 4 million traps are used for 40 nights during the summer, then 71,200,000,000 — more than *71 billion* nontarget insects — are needlessly destroyed in the U.S. each year by misinformed homeowners. If we substitute into our calculations the trap means obtained by Nasci *et al.* (1983) in Indiana (2163 insects during a 2 h trapping period; N = 10), this figure rises to nearly

Table 1. Seasonal totals of biting flies (in bold), predators and parasitoids (*italicized*), plus other taxa killed by electric insect traps at six sites in Newark, DE.

Order and Family	*No. Killed*	*% of Total*
Ephemeroptera	15	0.11
Dermaptera		
Labiidae	2	0.02
Psocoptera	14	0.10
Hemiptera		
Corixidae	10	0.07
Hebridae	2	0.02
Miridae	89	0.64
Nabidae	2	0.02
Lygaeidae	32	0.23
Rhopalidae	1	0.01
Cydnidae	14	0.10
Homoptera		
Cicadidae	33	0.24
Cicadellidae	2421	17.56
Flatidae	8	0.05
Acanaloniidae	1	0.01
Psyllidae	41	0.30
Delphacidae	1	0.01
Cixiidae	1	0.01
Aphididae	25	0.18
Thysanoptera	16	0.12
Neuroptera		
Corydalidae	1	0.01
Chrysopidae	8	0.05
Coleoptera		
Carabidae	661	4.79
Dytiscidae	21	0.15
Hydrophilidae	83	0.60
Staphylinidae	306	2.22
Lucanidae	1	0.01
Scarabaeidae	219	1.58
Buprestidae	3	0.02
Elateridae	46	0.33
Lampyridae	12	0.09
Cantharidae	104	0.754
Dermestidae	11	0.08
Anobiidae	30	0.22
Cleridae	4	0.03
Nitidulidae	27	0.20
Coccinellidae	15	0.11
Tenebrionidae	13	0.09
Mordellidae	10	0.07
Cerambycidae	11	0.08
Chrysomelidae	22	0.16
Curculionidae	7	0.05
Scolytidae	27	0.20
Diptera		
Tipulidae	223	1.62
Psychodidae	11	0.08
Culicidae	♂ 25, ♀ 18	0.31
Ceratopogonidae	♂ 30, ♀ 12	0.30
Chironomidae	4612	33.45
Scatopsidae	13	0.09
Simuliidae	1	0.01
Bibionidae	1	0.01
Mycetophilidae	34	0.25
Anisopodidae	13	0.09
Sciaridae	89	0.65
Dixidae	3	0.02
Cecidomyiidae	316	2.29
Stratiomyidae	5	0.04
Xylophagidae	1	0.01
Asilidae	1	0.01
Scenopinidae	1	0.01
Rhagionidae	2	0.02
Empididae	58	0.42
Dolichopodidae	70	0.51
Pipunculidae	1	0.01
Phoridae	12	0.09
Platypezidae	4	0.03
Otitidae	2	0.02
Tephritidae	2	0.02
Sciomyzidae	1	0.01
Ephydridae	8	0.05
Drosophilidae	7	0.05
Agromyzidae	14	0.10
Lonchaeidae	5	0.04
Lonchopteridae	8	0.05
Heleomyzidae	1	0.01
Sphaeroceridae	2	0.02
Anthomyiidae	28	0.20
Calliphoridae	17	0.12
Sarcophagidae	8	0.05
Tachinidae	16	0.12
Trichoptera	1597	11.58
Lepidoptera		
Microlepidoptera	1121	8.13
Tortricidae	19	0.14
Pyralidae	316	2.29
Geometridae	35	0.25
Lasiocampidae	3	0.02
Arctiidae	11	0.08
Noctuidae	64	0.46
Notodontidae	2	0.02
Epipyropidae	5	0.04
Yponomeutidae	10	0.07
Hymenoptera		
Braconidae	377	2.73
Ichneumonidae	77	0.56
Mymaridae	1	0.01
Perilampidae	1	0.01
Eulophidae	1	0.01
Encyrtidae	1	0.01
Pteromalidae	1	0.01
Torymidae	2	0.02
Eurytomidae	1	0.01
Chrysididae	3	0.02
Formicidae	84	0.61
Vespi	3	0.02
Halictidae	1	0.01

350 billion nontarget insects. We suggest, therefore, that while there is no evidence that zappers control nuisance insects, their effects may be anything but benign. Studies investigating the effects of insect defaunation on local ecosystems in general and on specialized insectivores such as bats and nighthawks in particular are needed to evaluate the ecological costs of zappers and other human activities destructive to insects. The results of our study indicate that entomologists, especially those active in extension, should be educating the public about the possible costs and lack of benefits from these gadgets.

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An assessment of mosquito breeding and control in 4 surface flow wetlands in tropical–subtropical Australia

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Abstract

In Queensland, Australia, the tropical–subtropical climate is ideal to promote macrophyte growth in surface flow wetlands, however there have been concerns that constructed wetlands are potential breeding sites for disease-bearing mosquitoes. The aim of this study was to assess whether mosquitoes were breeding in these constructed wetlands, and if so, where they breed, and what parameters might influence breeding: e.g. water quality; vegetation, or macroinvertebrate communities. A study of 4 surface flow constructed wetlands located in different climatic regions was undertaken. Mosquito larvae were sampled using 240 ml dippers and macroinvertebrates using dip nets. The wetland with the greatest biodiversity of macrophytes and macroinvertebrates had the least number of mosquito larvae (< 1 % of all dips). Samples with most mosquito larvae occurred amongst dense mats of *Paspalum* grass or dead *Typha*. Despite the presence of larvae in some parts of these wetlands very few late instars or pupae were found, i.e. completion of the mosquito life cycle to adult mosquitoes was unsuccessful.

This study has shown that the presence of mosquito larvae can be minimised by increasing macroinvertebrate biodiversity, by planting a variety of macrophyte types and species, excluding aggressive plant species, and maintaining at least 30% open water. Macroinvertebrates are probably a crucial factor in the control of mosquito larvae ensuring that predation of the early instars prevents or limits the development of pupae and the emergence of adults.

Keywords:
Constructed wetlands, macrophytes, macroinvertebrates, mosquitoes, predation.

Introduction

An awareness of healthy waterways and water re-use have created the need to develop and implement wastewater management strategies which are economically and ecologically sustainable. Constructed wetlands are an excellent option for water quality improvement particularly where land is available. Constructed wetlands for wastewater treatment are designed as ecological systems. Water quality enhancement is achieved through a combination of physical, chemical and biological processes facilitated by the plants and micro-organisms. Freewater Surface Flow (FWS) constructed wetlands are similar to many natural wetlands with plants being the most conspicuous feature. As ecological systems they support a variety of living organisms: plants, algae, macroinvertebrates, vertebrates, and a plethora of micro-organisms. However, the plants and animals living in FWS wetlands must be adapted to permanent flooding and often eutrophic water quality.

In Australia surface flow constructed wetlands for the treatment of municipal wastewater and urban stormwater are multi-functional, providing wildlife habitat, landscape enhancement, water storage and flood mitigation (Greenway and Simpson 1996; Greenway 2000). Despite these benefits to wildlife and humans, concerns have been raised by government authorities and the community that surface flow wetlands may be potential breeding sites for disease-bearing mosquitoes (NHMRC 1998, QDNR 2000). These limitations are also recognised by the USEPA 2000: "While a constructed wetland's attractiveness to wildlife may be regarded as a benefit to the human community, the potential for breeding mosquitoes can be an obstacle to permitting, funding, siting of a constructed wetland" p.17. This view is also shared by entomologists (Walton *et al.* 1998, Walton 2002, Russell 1999). Medical entomologists have claimed that constructed wetlands are directly responsible for an increased risk of disease due to an increase in mosquito breeding habitat, however not all species of mosquito are disease vectors or nuisances. Most mosquitoes are opportunistic breeders and if a suitable body of water is available they will deposit eggs. However, mosquito larvae are an integral component of aquatic food webs (US-EPA 2000, Greenway and Simpson 1996, Greenway 2000). Thus, the critical and significant issue is whether the larvae survive and whether adult mosquitoes emerge from pupae. It is the authors' view that if constructed wetlands are designed to function as wetland ecosystems with a diversity of aquatic organisms, then it is likely that the predator/prey mix would control mosquito breeding. If health risks can be avoided, this will increase public acceptance of the value of constructed wetlands for the treatment of wastewater. This is of particular significance to the wastewater industry since in order to promote the concept of constructed wetlands they must also demonstrate that risks of mosquito borne disease is minimal.

Observations by the first author indicated that mosquito breeding was not a problem in the 9 pilot scale wetlands in Queensland (Greenway and Woolly, 1999); however this needed to be quantified. A study commenced in November 1999 to investigate whether mosquito breeding was occurring in four surface flow constructed wetlands. The aim of the study was to determine if these constructed wetlands provided a suitable habitat for successful larval development of mosquitoes, and to try and identify which factors they either prevent or encourage larval development.

Methods

Site description — wetland design and water quality

Four surface flow constructed wetlands at different geographical locations with different climatic conditions were selected for this study. Cairns, coastal northern Queensland (tropical–wet); Blackall, inland central Queensland (arid); Cooroy (Noosa Shire), south east coastal Queensland (subtropical–wet); and Rosewood (Ipswich City Council) inland south east Queensland (subtropical–dry).

The Cairns Wetland was constructed in 1994 and was originally band planted with a diversity of species *Typha* spread rapidly and now covers 60% of Channel 1; 100% of Channel 2 and 70% of Channel 3; the remainder is predominantly *Eleocharis*; water depth is 40–50 cm. It receives secondary effluent after passing through an oxidation ditch. Water quality is low in ammonia but high in phosphorus (Greenway and Woolly, 1999; 2001).

The Blackall Wetland was constructed in 1993 and planted with 6 local species. The original depth of the channels was 50 cm, however in 1999 the channels were deepened to 1.5 m with steep vertical banks (along one or both sides), except at the inlet and outlet sections. *Typha* and floating rafts of *Paspalum distichum* now dominate these shallow areas, however 60–95% of the channels are open water. Channel 1 is 95% open water with a narrow band of *Typha* at the inlet and outlet section and along the perimeter on one side. Channel 2 is 75% open water with *Typha* at inlet and outlet sections and floating rafts of *Paspalum distichum* and *Persicaria attenutum*. Channel 3 is 60% open water; 30% is a dense raft of *Paspalum* and *Persicaria*, and 10% *Typha*. Channel 4 is 95% open water with small patches of *Typha* and isolated clumps of *Paspalum*. It receives secondary treated effluent (Greenway and Simpson, 1996; Greenway and Woolley, 1999).

The Cooroy Wetland near Noosa is the largest wetland. The first wetland was built in 1995 and consists of 3 large cells linked by pipes. The second wetland of similar dimensions was built in

1999. Each cell is separated by septa into sections producing a sinusoidal flow path. Cell 1 is shallow (20–40 cm) throughout. Cell 2 and Cell 3 each have a deep pond (1–2 m) in the first section (30 m length), the remainder is shallow. The wetland supports a diversity of plants. The secondary effluent is treated by a trickling filter followed by alum dosing (to remove phosphate by precipitation), it then flows into an open water lagoon prior to release into the wetlands.

The Rosewood Wetland near Ipswich was built in 1995 and consists of 4 rectangular cells. Cell 1 is a deep open water lagoon (150 cm); Cells 2 and 3 are surface flow wetlands dominated by a wide band of *Typha* around the periphery, with a deeper open water pond in the centre; Cell 4 is a subsurface flow wetland planted with *Phragmites australis*. The wetland receives primary settled effluent which flows into Cell 1 where further settling takes place, before discharge into the vegetated cells.

Water depth, temperature and dissolved oxygen were recorded at each sampling station. Annual temperatures ranged from 15°C to 27° at Cooroy and from 22° to 30° at Cairns. Dissolved oxygen was highly variable — varying seasonally, diurnally, with depth and vegetation. Open water areas had the highest DO (up to 16 mg/L). Cairns recorded 13 mg/L amongst submerged pond weed (*Ceratophyllum*) and algae. DO decreases with depth to negligible values amongst or beneath dense or dead vegetation.

Field sampling

At each site a minimum of 3 sampling stations were established in each channel or cell. Where possible these were located where there was vegetation. At Blackall, Rosewood and Cooroy open water sections and storage lagoons were also sampled. Vegetation in the wetlands was recorded according to species, type and percentage cover.

Mosquito larval distribution was assessed by taking 10 surface dips, using a 240 ml dipper, at each station. Mosquito larvae numbers were counted and for each dip categorised into: no larvae (0), 1–10 larvae, 11–40 larvae and greater than 40 larvae. In addition, the developmental stage of the larval instars — 1st, 2nd, 3rd, 4th and pupae were noted. Where possible the larvae were identified to species. Dip nets were used to sample the aquatic biota by swoops conducted within a 2 m radius of each station. Water depth, temperature and dissolved oxygen were recorded.

Results

Macrophytes

Table 1. Macrophyte species richness in the 4 surface flow constructed wetlands

Macrophytes	Cooroy	Cairns	Blackall	Rosewood
Emergents	23	10	4	3
Attached with floating Leaves	7	8	1	1
Free floating	4	6	3	3
Submerged	4	1	–	–
Total species	38	26	8	7
% Cover	70%	95%	10%	95%

A summary of the categories of macrophyte types and the overall percentage cover vegetation is given in Table 1. For details of plant species refer to Greenway *et al.* 2002.

Cooroy had the greatest species richness with a total of 38 species. Of the emergent macrophytes the most abundant species were the sedges *Baumea articulata, Baumea rubiginosa, Lepironia articulata, Schoenoplectus validus* and the flowering *Phylidrum lanuginosum*. The diversity of species can be attributed to direct planting, natural colonisation and active management to remove invasive weed species. The shallow depth 20 cm for most cells is optimal for the growth of these emergent species. The deep cells (1.5–2 m) were suitable for water lilies and submerged species.

Cairns had 26 species, however *Typha* was the dominant species forming dense monospecific stands of live and dead shoots in all 3 channels. Only 2 channels had sections which had not been completely colonised by the spread of *Typha*. In Channel 3 these sections were dominated by *Eleocharis sphaceolata;* in Channel 1 there were sections of *Eleocharis, Schoenoplectus* and *Paspalum. Ceratophyllum* occurred beneath the *Schoenoplectus.*

Rosewood was also dominated by *Typha*, which formed dense stands of live and dead shoots, isolated clumps of *Baumea articulata* extended into the deeper centre of Cell 2 and clumps of *Cyperus* sp occurred at the margins. The channels at the Blackall wetland were mostly free of vegetation except for the shallower inlet and outlet sections which contained *Typha*. Dense mats of water couch *Paspalum distichum* and knotweed *Persicaria attenuatum* had formed in sections of Channels 2 and 3.

Macroinvertebrates

A summary of the total number of macroinvertebrates taxa in the major classes is given in Table 2 — For a complete list of these taxa identified to family, genus and species refer to Greenway *et al.* 2002. Cooroy had the greatest species richness, in particular the larval stages of dragon flies (Epiproctomorpha), damsel flies (Zygoptera) and caddis flies (Trichoptera), and pond snails. Cooroy and Rosewood both had large numbers of water beetles. Macroinvertebrate sampling at Cairns was limited to three collections, hence species richness at Cairns is much lower than expected.

Table 2. Major macroinvertebrate taxa present in the four wetlands

Taxa	Cooroy	Cairns	Blackall	Rosewood
Gastropoda (snails)	8	2	1	
Annelida (worms/leeches)	3	1	5	2
Crustacea (copepods, ostrarods)	5	4	3	3
Ephemeroptera (May flies)	3	3	1	
Epiproctomorpha (dragonfly)	17	8	3	
Zygoptera (damsel fly)	8	4	2	1
Hemiptera (water bugs)	8	6	9	4
Diptera (flies/mosquitoes)	9	11	5	8
Coleoptera (water beetles)	19	4	8	18
Trichoptera (Caddisfly)	7		1	
Total taxa	87	43	38	36

Mosquitoes

Ten species of mosquito larvae were identified: *Anopheles annulipes*, *Culex annulirostris*, *C. australicus*, *C. gelidus*, *C. halifaxii*, *C. squamosus*, *Culicinae* sp, *Uranotaenia* sp. *Verrallina carmenti*, *V. lineatus*. All species were found at Cairns. Only *C. annulirostris* is a known vector of Ross River virus. Larvae of this species and *C. annulipes* were found in all four wetlands.

A summary of the relative abundance of mosquito larvae based on the percentage number of dips containing either no larvae, less than 10 larvae, 11–40 larvae or more than 40 larvae are given in Table 3. At Cooroy less than 1% of dips in Cells 2 and 3 contained any larvae. 2% of dips in Cell 1 contained larvae and these were only found at the sampling station with dense *Phragmites* and overgrown mats of *Paspalum* grass. At Blackall all sampling stations in Channel 1 were open water and only 0.5% of dips contained larvae. In Channels 2 and 3 the mid station was open water but the other two stations contained either *Typha* or isolated *Paspalum* clumps; the dips with > 40 larvae were sampled from within the *Paspalum* clumps. Channel 3 had the greatest cover of vegetation (40%) — with dense stands of *Typha* and floating mats of *Paspalum* and *Persicaria*, 16% of dips contained larvae. All dips with larvae were sampled at the station with the floating mats of vegetation.

At Cairns 10–20% of the dips contained larvae. Only 1 dip contained 11–40 larvae and 1 dip contained > 40 larvae and these both occurred on the same sampling day. All samples were taken at stations with dense stands of live and dead *Typha*.

Table 3. Relative abundance (% of dips) of mosquito larvae

Location		# dips	No larvae	< 10 larvae	11–40 larvae	> 40 larvae
Cooroy:	Cell 1	210	98%	2%	–	–
	Cell 2	210	99.5%	0.5%	–	–
	Cell 3	210	99%	1%	–	–
Cairns:	Channel 1	40	80%	17.5%	–	2.5%
	Channel 2	40	87.5%	12.5%	–	–
	Channel 3	40	80%	17.5%	2.5%	–
Blackall:	Channel 1	220	99.5%	0.5%	–	–
	Channel 2	210	95%	2%	–	3%
	Channel 3	190	84%	14%	2%	–
	Channel 4	200	96.5%	3.5%	–	–
Rosewood	Cell1 pond	200	95%	5%	–	–
	Cell 2 Typha	170	68%	25%	6.5%	0.5%
	Cell 3 Typha	140	54%	40%	6%	–

At Rosewood the open water settlement pond (Cell 1) recorded the least larvae (5% of dips), whereas the vegetated cells had the highest number of larvae: 32% of dips in Cell 2 and 46% of dips in Cell 3. Most of these larvae were concentrated in the dense stands of *Typha* with dead submerged stems and leaves, around the shallow edge of the cells. At each of the 4 wetlands 70–90% of the larvae found were early instars (1st and 2nd stages) and less than 1% were pupae.

Discussion

Macrophytes facilitate water treatment in constructed wetlands but they are also essential for ecosystem functioning. Sustainable populations of organisms are dependent upon habitats that provide the attributes for a species complete life history. Wetland plant diversity is important for determining macroinvertebrate associations (De Szalay and Resh 2000) and wildlife diversity (Knight *et al.* 2001) because of the creation of habitats and food resources. Wetzel (2001) noted that the most effective wetland ecosystems "are those that possess maximum biodiversity of higher aquatic plants and periphyton associated with the living and dead plant tissue". In constructed wetlands macroinvertebrate biodiversity is also enhanced by good water quality — secondary or tertiary treated effluent, and aerobic conditions.

Our study of four surface flow constructed wetlands found that the Cooroy wetland with shallow marsh and deeper ponds had the greatest species richness of macrophytes (38 species) and macroinvertebrates (90 taxa) and the lowest occurrence of mosquito larvae (< 1% of dips). The Cairns and Rosewood wetlands dominated by dense monospecific stands of *Typha* had fewer macroinvertebrate taxa (47 and 38 respectively) and a higher proportion of mosquito larvae (20% of dips at Cairns and 40% of dips at Rosewood). The Blackall wetland was mostly open water with small stands of *Typha* and sections with floating mats of water couch (*Paspalum*) and 41 macroinvertebrate taxa were found. Less than 0.5% of dips in the open water channels contained mosquito larvae whereas 16% of dips from amongst the *Paspalum* had larvae. A marsh with a diversity of macrophytes appears optimal for macroinvertebrates biodiversity and the control of mosquito larvae by predation.

Dense stands of *Typha* with an accumulation of submerged dead stems and isolated pockets of water are suitable for mosquito breeding. Similarly dense floating mats of *Paspalum* grass and *Perscicaria* are also suitable for mosquito breeding but of limited habitat value for many macroinvertebrates due to the lack of swimming space and low dissolved oxygen. Mosquito larvae are surface breathers and can survive in anaerobic conditions, however many aquatic macroinvertebrate predators are also surface breathers, e.g. notonectid bugs, water beetles, or surface predators, e.g. pond skaters. Predation is best avoided if the mosquito larvae can isolate themselves from predator access. Orr and Resh (1992) found that dense beds of *Myriophyllum aquaticum* were a primary habitat for *Anopheles* larvae where they survive in microhabitats. Walton (2002) noted that in the arid south western United States constructed treatment wetlands can increase mosquito production if there is poor water quality and dense

coverage of submerged dead vegetation. An abundance of notonectids in the settlement pond (Cell 1) at Rosewood probably accounted for low numbers of mosquito larvae.

Despite the presence of more mosquito larvae at Rosewood and Cairns the low numbers of $3^{rd}/4^{th}$ instars and pupae suggests predation. Walton and Workman (1998) attributed the lack of late instars in a Californian constructed wetland to invertebrate predators, mostly notonectid bugs.

There is limited published material on the ecological characteristics of constructed treatment wetlands, in particular with respect to aquatic invertebrates. Knight *et al.* (2001) summarised some of the key finding of the North American Treatment Wetland Database (NADB v 2.0) with respect to quantitative data on habitat, wildlife, human uses and ecological risks. Martin *et al.* (2001) conducted an ecological survey of a cypress-gum swamp forest in Florida that had been receiving secondary treated effluent since 1984. In addition to biological monitoring for vegetation, benthic macroinvertebrates and fish, they also monitored mosquito larvae and pupae in the summer months from stations that were 70% vegetation cover. Although direct quantitative comparisons cannot be made with the present study due to different methods of recording larval abundance they noted that "the number of immature mosquitoes collected at each station was typically low". Using twenty 450 mL dippers at each station they recorded an average number of larvae between 143–527/m^3, i.e. 0.64–2.4 larvae per dip. They also noted that macroinvertebrate density measured using Hester-Dendy samplers was also low. They attribute low dissolved oxygen as the most significant factor affecting macroinvertebrate populations. The wetland supported a diverse and abundant fish population which Martin *et al.* suggest "appears to provide a significant control on mosquito populations" p.323.

The exotic mosquito fish *Gambusia holbrooki* was found at Cooroy and Blackall where it was deliberately introduced to control mosquitoes. However, the effectiveness of *Gambusia* in the control of mosquito larvae in Australian wetlands and waterways has not been scientifically proven with mosquitoes only making up a small part (< 10%) of their diet. "Several authors have observed that gambusia may actually encourage mosquito populations by preying on their invertebrate predators" (NSW–NPWS 2002). As with most introduced species *Gambusia* is competing with native species for food and habitat. The Cairns wetland supported a large tadpole (and frog population) probably due to the absence of *Gambusia*.

Walton and Workman (1998) conducted a comparative study of mosquito larvae and macroinvertebrates in two structurally different constructed wetland cells. Design 1 consisted of a shallow (0.5 m) densely vegetated marsh (*Schoenoplectus californicus*); design 2 had shallow inlet and outlet marshes separated by a section of deeper (1.2 m) open water. Larvae were sampled in the vegetated sections of both designs. Larvae abundance was higher in design 1 and contained proportionately more later ($3^{rd}/4^{th}$ stage) larval instars. Average larval numbers ranged from 10 per dip (400 ml) in early summer to less than 1 per dip in late summer in design 2, and from 40–60 per dip in early summer, to 2–5 per dip in later summer, in design 1. Predatory macroinvertebrates (notonectids, dragonfly nymphs, beetle larvae) were more abundant in early summer in design 2. Differences in larval abundance were attributed to larger populations of predators in design 2 facilitated by habitat preference for open water — particularly for notonectids.

Our study has also shown that maximum biodiversity of macrophytes and macroinvertebrates, and minimal mosquito larvae survival can be achieved by having a combination of shallow marsh vegetation (20–40 cm depth) with no more than 70% plant cover and deeper (1–1.5 m) open water ponds. Aggressive plant species such as *Typha* and *Phragmites* should not be planted unless they are managed to prevent spreading and the build up of submerged layers of dead leaves and stems. Harvesting may be a management option. Plant species that produce thick floating mats such as *Paspalum distichum* (water couch) and *Persicaria* sp (knotweeds) should also be discouraged as they cause anaerobic conditions in the underlying water column, provide pockets of stagnant water for mosquitoes to breed and prevent predator access. Greenway (2003) has reviewed the suitability of macrophytes in surface flow wetlands in tropical–subtropical climates, with genera such as *Schoenoplectus, Lepironia, Baumea, Phylidrum, Bolboschoenus* providing alternatives to *Typha* and *Phragmites*.

The management of wastewater must be consistent with protection of risks to human and animal health from mosquito-borne disease. Constructed wetlands should be designed to

optimise wastewater treatment and ecological benefits to wildlife while minimising the potential for nuisance conditions such as mosquito breeding. Invertebrate predation is an important method for mosquito control. The value of constructed wetlands as low-cost alternatives to traditional wastewater treatment facilities has even greater significance in populated developing countries such as Africa and India where there is limited wastewater treatment. Constructed wetlands in these countries offer an economical solution to wastewater treatment providing they can be assured that other health risks such as mosquito-borne diseases are eliminated.

Conclusion

This study of four surface flow constructed wetlands in Queensland, Australia, has shown that the Cooroy wetland has the greatest species richness of macrophytes and macroinvertebrates and the lowest occurrence of mosquito larvae, suggesting that vegetation diversity (type, species and cover) is a significant factor in the control of mosquito breeding in constructed wetlands. Both the Cairns and Rosewood wetlands were dominated by dense monospecific stands of *Typha* and had a large build-up of dead organic matter — stems and leaves — beneath the surface. These woven mats of leaves formed small isolated pockets of water suitable for mosquito larvae development. These isolated pockets make it harder for predator access as well as having low dissolved oxygen concentration associated with microbial decomposition of dead organic matter. At Blackall mosquito larvae were of greatest occurrence amongst the dense mats of *Paspalum* grass — again the interwoven stems and roots restricting predator access, but very few larvae occurred in the open water sections.

Our study showed that although mosquitoes lay eggs in the wetlands and these hatch into larvae, very few larvae are likely to emerge as adults due to predation of the early instars. There needs to be wider recognition that mosquito larvae are an integral component of wetland ecosystems, and providing ecosystem functioning is maintained, then predator–prey relationships will ensure the control of mosquito breeding. Wetland design and maintenance is necessary to ensure wetland ecosystems support a diversity of macroinvertebrate predators. In Australia the introduction of the mosquito fish *Gambusia* is not recommended. Public acceptance that constructed wetlands are multi-functional systems providing benefits for human use, wildlife and downstream aquatic ecosystem health, will ensure that these treatment systems become an integral component of our urban landscape.

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THE ECHOLOCATION OF FLYING INSECTS BY BATS

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When bats are hunting insects they adjust the pattern and tempo of their high frequency orientation sounds in a way that seems quite appropriate for active echolocation of small moving targets but distinctly unsuited for the passive detection of insects by listening for their flight sounds. (Griffin, 1953; 1958). The most obvious change is a marked rise in the pulse repetition rate just as the bat closes in on its prey. For example, *Eptesicus fuscus* often emits only four or five pulses per second in straight cruising flight, with silent intervals of 200 milliseconds between them; but during insect pursuit the same bat may shift to a "buzz" in which the pulses are separated by only five milliseconds. Similar flight patterns and vocal responses can be elicited by tossing into the vicinity of a hunting bat imitation insects such as pebbles or plagets of wet absorbent cotton. What little sound these make can scarcely resemble that of a real insect.

Yet this type of evidence is indirect and not wholly satisfactory, since some bats certainly do respond to the sounds made by buzzing insects (Moehres, 1950; Kolb, 1959). Studies of insect pursuit behaviour in the laboratory have recently enabled us to make four significant extensions of earlier studies under natural conditions: (1) a more detailed description of the bats' hunting tactics, (2) measurements of the high rates at which small insects are captured, (3) estimates of the minimum distances at which they are detected, and (4) selective masking experiments with low and high frequency noise which demonstrate conclusively that at least under some circumstances insects such as *Drosophila* are intercepted by echolocation.

It is convenient to distinguish three phases of insect hunting behaviour which appear always to be present with varying degrees of distinctness in bats of the family Vespertilionidae. The first or *search phase* is fairly straight flight in which the pulse repetition rate is relatively low, although the bat is evidently ready to attack flying insects. The second or *approach phase* begins when the bat first reacts to an insect, either by turning towards it, by increasing the pulse repetition rate, or both. Here the interval between pulses shortens progressively though often irregularly, as the bat apparently locates its prey more accurately and flies toward it. A *terminal phase* ensues when the bat is fairly close to the insect and emits a burst of pulses at a very high rate, the buzz. A similar buzz is emitted on dodging small wires or just before landing, but the buzz of an insect catch seems typically to last longer and to include more closely spaced pulses. In *Myotis lucifugus*, the species we have studied most thoroughly, the search phase involves pulse-to-pulse intervals varying from about 50 to 100 milliseconds or more; in general the larger the room the longer the intervals in this phase. The approach phase has intervals between about 50 and 10 milliseconds, and in the buzz the pulses are separated by only 4 to 7 milliseconds. Intervals of more than 50 milliseconds are thus characteristic of cruising or search, 50 to 10 milliseconds approach, and less than 10 milliseconds the terminal phase of very close pursuit or actual capture of the insect.

Methods

Although small insects are apparently detected and pursued one at a time, the bats we have studied were much more likely to begin active hunting when large numbers of insects were on the wing. Even at best we have found that only a small fraction of the *Myotis lucifugus* released in rooms filled with flying insects undertake serious hunting; many will slowly starve and would doubtless die eventually in a room where other individual bats catch hundreds of insects every day. Two or three sessions of a half-hour or less on different days are usually sufficient to find the good catchers, provided that the bats are wide awake, warm, and active when tested. The 90 per cent. or more that did not show signs of hunting after several such sessions were released.

A good catcher identifies itself by making obvious interception manoeuvres, turning sharply, suddenly climbing or diving for a few inches, or merely by pulling up abruptly in the midst of fairly straight flight. Often the interfemoral membrane can be seen to turn upwards and forward, and the head may be thrust down into it very rapidly and out again. If the room is quiet, one can hear with the unaided ear the

faint audible clicks which accompany each pulse of high frequency sound during the search or approach phases. The buzz accompanying the actual catching manoeuvre is usually more clearly audible. With adequate lighting the actual capture of insects may be seen, but this has been much less obvious than the flight manoeuvres or the faint audible buzzes.

We have studied insect hunting in three different rooms, all about 8 feet high. The first, at the Vero Beach Laboratory of the Florida State Board of Health, was 8 ft. × 16 ft., the two used later in Cambridge, Massachusetts, were 12 ft. × 32 ft. and 11 ft. × 16 ft. All of these rooms had walls which reflected high frequency sound quite well, and in all cases the bats must have received strong echoes from walls, ceiling, and floor along with the much fainter echoes from their insect prey. This "clutter" may well have been what discouraged most bats from hunting indoors.

Sufficient numbers of insects might be obtained by light traps or other means of attracting wild insects, but we have reared mosquitoes and fruit flies by standard methods (Galtsoff, *et al.*, 1959). It was necessary to stir the insects into flight every few minutes in order to elicit continuous hunting. The actual densities of flying insects varied widely, both in space and time; but the great majority of catches were probably made at densities of no more than three or four insects per cubic foot.

Since insect capture by bats is a rapid process, and since the small insect is often very difficult to see at all, we were anxious to record the hunting behaviour by single photographs and motion pictures. With single photographs, even when the insect as well as the bat is shown, it is difficult to be certain what part of a complex sequence of events is recorded. Multiple flash stroboscopic photographs have been very useful, and at a flashing rate of five to ten per second the successive images of a flying bat are sufficiently separated, except in turns or hovering manoeuvres. Insects can be photographed better with multiple than with single flashes, because a row of specks equal in number to the flashes is less likely to be confused with dust on the film. We have used both still and motion picture cameras in pairs, spaced 0·5 or 1 metre apart, in order to obtain more accurate information about the positions of bat and insect, by simultaneous cross bearings.

In many experiments a tape recording was made of the bat's orientation sounds with a plastic dielectric condenser microphone (Kuhl *et al.*, 1954), a suitable amplifier, band pass filter, and an Ampex tape recorder. Each flash of a strobe light can easily produce a recognizable electrical signal on the tape, so that when oscillograph pictures of the bat's sounds were later prepared from the tape recordings it was quite feasible to identify each flash and determine its time of occurrence relative to the recorded sounds from the bat.

Illumination and background surface are of great importance because bats are very dark and tend to blend into most backgrounds if photographed by reflected light. It is relatively easy to obtain silhouettes of flying bats against a white background, and if the background is marked off with a grid of contrasting lines, the bat's position can be determined with one camera by making parallax corrections (Grinnell & Griffin, 1958). If two lights are employed, the separation between the two shadows of a bat can be used to determine its distance from the wall. We were not able to use this method to photograph mosquitoes or *Drosophila*, however, and turned instead to a very dark background of black velvet with lateral illumination either by flood lamps or an electronic flash. By careful arrangement of the lamp or lamps it was possible to photograph both bats and individual fruit flies on 16 mm. film. The flies appeared only as specks, but in many cases a careful study of the film with a time motion study projector permitted unequivocal identification of the flies by their characteristic movement. Catches were recognized by the approach of a bat to a particular fly followed by the disappearance of the latter as the bat flew on. A dust speck could easily be mistaken for a single image of a fly, but dust varied randomly from frame to frame while flies remained in approximately the same place or moved progressively. Furthermore dust specks were not intercepted by the bats.

1. Hunting Tactics

Most of the pursuit and interception manoeuvres requires less than one second and are extremely difficult to observe directly. We therefore photographed the bats and insects and recorded simultaneously the pattern of the orientation sounds. Each of the catches for which full or fragmentary sound and position records were obtained was preceded by the customary search and approach phases defined above, although there were some catches or appararent catches of mosquitos without a full-fledged buzz. In such

cases the pulse-to-pulse interval dropped from about 90 milliseconds in the search phase only to about 40 or 50 milliseconds. This shift from search to approach phases provides the best indication yet available of the distance by which a bat has already detected some small object, such as a wire or an insect. In two mosquito catches for which excellent records were obtained there was a clear drop in this interval at about 100 centimetres and at about 30 centimetres respectively from the probable position of the mosquito. That is, the interval showed a marked drop at these distances from the spot where, a fraction of a second later, the bat was to emit its sharp buzz. Mosquitos can therefore be detected at appreciable distances, but further discussion of this topic is best deferred to Section 3.

The manner in which the frequencies within each pulse varied during these catching manoeuvres is also of interest. In the search phase, before any apparent reaction to the insects, the pulses were about two milliseconds in duration, the intervals between pulses were roughly 80-100 milliseconds, and the frequency dropped in each pulse from about 75-85 kilocycles at the start to 37-45 kilocycles at the end. This is the typical octave of frequency sweep in a *Myotis* pulse, although the actual frequencies were slightly lower than on some other occasions. During the two pronounced buzzes mentioned above the pulse durations fell to 0·5 milliseconds, or sometimes a little less. The estimated frequencies within these and other very short pulses ranged from about 25 to 30 kilocycles. Our recent and technically much improved records thus require an important modification of the first description of frequency patterns during insect catching (Griffin, 1953). It is now clear both for *Myotis* and *Eptesicus* during the pursuit of real and imitation insects that in the terminal buzz the frequencies are distinctly *lower* than in the search phase (previously referred to as "cruising", an appropriate term for *Eptesicus* under natural conditions but much less apt for *Myotis* catching insects at high rates in a small space).

During the pursuit of an insect the actual lengths of the pulses of sound in air drop, in proportion to the pulse duration, from about 70 cm. to 15 cm. The distance travelled by the bat between pulses varies with its flying speed, but before and just after detection (indicated by the drop in pulse-to-pulse interval) the bats were flying at 2 to 3 metres per second, or 2 to 3 millimetres per millisecond. One of the longer intervals thus represents 200-300 mm. of travel, while during the two clearest buzzes the bat's speed was about 70 cm./second or 0·7 mm./millisecond. Since one pulse was emitted every 5 to 7 milliseconds, the bat was now travelling only about 3·5 to 5 mm. between pulses. The velocity of sound in air is about 340 mm./millisecond, so that the wave lengths of the orientation sounds were about 4 to 8 mm. in the search phase, but increased during the buzz. The catching manoeuvres and their relationship to the pattern of the buzz will be described in more detail in a later paper.

2. Rates of Insect Capture

a. Mosquitos

In April, 1958, at the invitation of E. T. Nielsen several bats were brought to his 8 ft. × 16 ft. flight chamber at Vero Beach, Florida, which contained initially about 2,000 mosquitos (*Culex quinquefaciatus*). Several *Myotis lucifugus*, one *M. subulatus*, one *M. subulatus leibii*, two *Pipistrellus subflavus*, and two *Plecotus rafinesquii* were set free in this room for 10-15 minutes at a time, and certain of the *Myotis* hunted actively. These were left in the chamber overnight, and in the morning the mosquito population had fallen to roughly 200-300. Although none of the *Pipistrellus* or *Plecotus* was ever observed to hunt actively, a few of the *Myotis* were so successful that we could estimate the rate of insect capture not only by counting audible buzzes, but also by measuring the rate at which the bats gained weight (See Table I). Many factors might reduce the weight gain—evaporation of water from skin or lungs, urination or defaecation—but since the bats were closely watched we could be sure that they did not eat or drink anything but insects caught on the wing. Their gain in weight thus represents a conservative minimum estimate of the weight of insects captured.

It was not practicable to restore the population of mosquitos to its original level during these experiments, and on some of the subsequent days only a few hundred were present in the flight chamber. Yet in eight cases listed in Table I accurate weighing demonstrated high rates of mosquito catching. On other occasions the same bats gained very little, or even lost weight while flying in the same chamber. In these cases observation and listening for the faint audible components of the buzzes confirmed that they were hunting much less actively. The mosquito population was so low on 5th April

Table I. Rates of Mosquito Catching by Bats at the Vero Beach Laboratory, April 1958. The average weight of six of the mosquitoes used (*Culex quinquefasciatus*) was 2·2 milligrams.

Bat	Date	Duration of flight (minutes)	Weight gain (mg.)	Mosquitoes caught per minute
M. lucifugus Number 5 (Wt. 5·7 gr.)	April 1	30	350	5·3
	April 4	31	100	1·5
	April 4	9·5	73	3·5
	April 5	15	55	1·7
M. lucifugus Number 7 (Wt. 5·7 gr.)	April 2	10	125	5·7
M. subulatus leibii (Wt. 3·7 gr.)	April 2	21	77	1·7
	April 4	24	264	5
	April 5	15	312	9·5

that it was supplemented by releasing in the same room several dozen large crane flies (*Brachryprenna*). These weighed about 19 mg., but the bats usually dropped the legs and wings uneaten, and the bodies weighed only about 12 mg. During the 15 minutes when the *M. subulatus leibii* was observed to gain 372 mg. it was clearly observed to catch no more than 4 or 5 crane flies along with many mosquitos. It seems most likely that less than 60 mg. of its 372 mg. weight gain consisted of the crane flies, so that its rate of mosquito catching is listed in Table I as 312/15 or 21 mg. per minute. This bat was thus catching about ten mosquitos per minute or one every six seconds.

b. *Drosophila*

During the spring and summer of 1959 large numbers of *Drosophila* were reared in an 11 ft. × 16 ft. flight chamber and out of many *Myotis lucifugus* which were allowed to fly in this chamber, four, designated below as TR, S, B and TL, began active catching immediately when first allowed to fly in this room. The fruit flies were unfortunately not maintained in pure culture, but only two species were present, *Drosophila robusta* weighing 3 mg. on the average, and *D. melanogaster* with an average weight of 0·6 mg. On several occasions certain of these bats gained as much as 29 mg./minute while catching only fruit flies in this room. Sustained catching rates of 20 fruit flies per minute were observed, and two flies were often taken within one second (For details see below, especially Table III). These rates of weight gain were in satisfactory agreement with the number of visible pursuit manoeuvres and audible buzzes which were often noticeable every few seconds, and are also quite similar to the estimates reported by Gould (1955; 1959) for the same species hunting under natural conditions.

3. Distances at which Insects are Detected

a. Procedures

This subject was studied by a minor modification of the photographic method used to measure the distance at which small wires are detected (Grinnell & Griffin, 1958). Bats and fruit flies were photographed in an 11 ft. × 16 ft. flight room with bright lateral illumination against a dark velvet background with a 16 mm. Auricon sound-on-film camera. The bat's orientation sounds picked up by the microphone were rectified into low frequency clicks by a detector circuit, and supplied to the input of the sound camera. Each bat pulse was registered, on the same piece of film as the pictures, as a click which contained no information about the original frequency, only rough information about pulse duration and intensity, but accurate information about pulse repetition rate or interval between pulses. This camera operates at 24 frames per second, with its shutter open about 1/50th second. While the images were small and slightly blurred by the bat's motion, they sufficed to show its position within one or two inches every 24th of a second. Actually the

position shown was the projection of the bat or fly against the opposite wall, and estimates of the distance between the two are subject to parallax errors in either direction. The apparent distance was greater than the actual distance when bat and fly were equally far from the camera, but less when one was behind the other. We could make only rough estimates of the bat's distance from the camera or the opposite wall, and hence we did not attempt to apply parallax corrections. But a long series of such single camera pictures allowed approximate estimates of the distance of detection and provided a most helpful background for more accurate determinations of bat and insect positions with paired cameras.

Detailed analyses were made of 34 catches or attempted catches by one *M. lucifugus*, S, 26 by another of the same species, B, and six by a *M. keenii septentrionalis*. In each of these 66 cases graphs were prepared showing, on a common time base, the pulse-to-pulse intervals, the vertical and horizontal positions of the bat and fly, and the apparent distance between them, that is, the distance between their images on the wall opposite the camera.

b. Special Aspects of Insect Pursuit Behaviour Relevant to Measurements of the Distance of Detection

Before proceeding to analyse these data on distance of insect detection it is important to review certain aspects of the bat's behaviour during these insect catching manoeuvres. In several cases the bat began its pursuit by taking off from the wall where it had previously been resting, and flew out to catch a fly which may well have been detected before take-off. In many other instances, however, the detection clearly occurred while the bat was flying; often many flies were caught during a continuous flight; and sometimes the bat did not land until hundreds of catches had been made. When starts from the wall were eliminated along with cases where the bat was turning away from the wall, catching another insect, or executing some other special manoeuvre at the start of the search phase, the pulse-to-pulse interval during search varied from 48 to 85 milliseconds, with the range from 50 to 60 milliseconds predominating. On the average the first clear drop below 50 milliseconds occurred about 0·5 second before the catch; and after that time almost every approach phase included a steady drop in interval. In all cases the actual detection must have taken place somewhat earlier than the vocal reaction of shortening the pulse-to-pulse interval. But since we have no way of estimating the bat's reaction time we will conservatively consider that detection occurred at the moment when the first pulse was emitted after an interval significantly shorter than that characteristic of the search phase.

The actual number of pulses emitted during the approach phase varied between 4 and 22; the average for the two *M. lucifugus* was 8·1 and for the *M. keenii* 10·6. These pulses are presumably used to track the moving insect. The terminal buzz contained anywhere from 3 or 4 to 15 or 20 pulses separated by 5 to 10 milliseconds. We have no way of determining whether the last pulse of the search phase served for detection, or whether several searching pulses at roughly 50-millisecond intervals were required. The number cannot be very large, however, because of the numerous cases described in the next section in which one catch was completed and a second detection and interception begun within a remarkably short period of time.

c. Double Catches

In six of the 66 cases selected for careful study there were two catches or attempted catches within less than one second. The actual period between catches was ¾ second for the *M. keenii*, ½ second for *M. lucifugus*, and three cases of about ½ second plus one of ¾ second for *M. lucifugus* B. In all cases the two flies were intercepted by distinct manoeuvres, and it seems probable that the second insect was located only after the first had been seized. (In one of these six cases the first fly was attacked but missed). Hence only a short interval of time and a few pulses are necessary for a successful search phase and detection. One of the clearest examples of double catches within one second is illustrated in Fig. 1. The positions of bat and fly were not clear until after the moment when the sound track first became adequate to show the tempo of the orientation sounds, but the latter part of the first approach phase was clearly photographed. The most significant aspect of this sequence is the time between the two catches. The next pulse to follow the first buzz came at a long interval (118 milliseconds) after the last pulse in the buzz. This is a common feature of these records, and it may well represent the time needed to remove the insect from interfemoral membrane where it is often "pouched" immediately upon capture or which it may strike before being seized in the teeth.

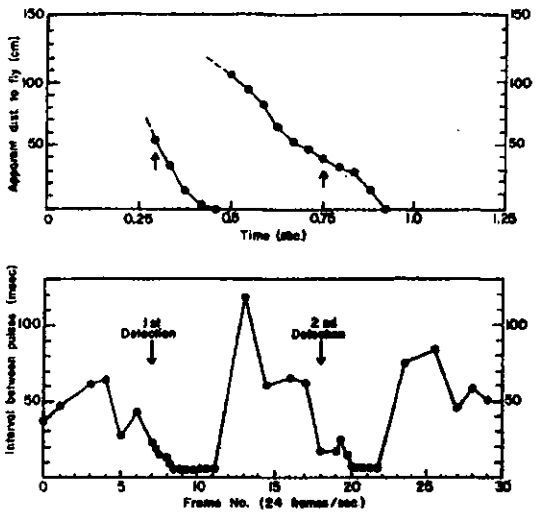

Fig. 1. A typical double catch within half a second. The upper graph shows as a function of time the apparent distance between the bat and the two *Drosophila*, that is, the separation between their images against the wall opposite the camera. Parallax errors may render these distances too high by 25 per cent. Each point on the lower graph shows on the same time base the interval separating one pulse of sound emitted by the bat from the previous pulse. Arrows mark the first vocal reaction to each fly, conservatively designed as the point of detection. During the two buzzes there were more pulses than could be plotted as separate points. Note that only four pulses were emitted in the search phase for the second catch (between 0.6 and 0.7 second).

Then three pulses were emitted at intervals of about 62 milliseconds, a value quite similar to the search phases just before and after the two catches. The next pulse came at an interval of only 17 milliseconds and clearly represented the beginning of the second approach phase. Only four pulses were emitted during the search phase between the two catches, hence the second fruit fly must have been detected by means of no more than four echoes. The reactions of these bats must thus be very rapid, and the information extracted from a very few pulses must suffice to locate the small target presented by a *Drosophila*.

d. Results Obtained with One Camera

Almost every one of the 66 cases of fruit fly catches analysed graphically provided a clear indication of the point at which the search phase gave way to the approach phase, thus demonstrating that the fly had already been detected. When the apparent distances of detection were averaged without parallax correction, the values were 73 cm. for the *Myotis keenii*, 55 cm. for *M. lucifugus* S, and 73 cm. for *M. lucifugus* B. The extreme values of these estimated distances of detection were 21 and 135 cm. Our next step was to average the individual values of pulse-to-pulse interval as a function of apparent distance from the fly. The mean value of the interval thus obtained showed an increase out to a distance of about one metre. This might be interpreted as evidence that detection occurs at this distance, but the flight patterns and other circumstances such as proximity to the walls differed so much in these several cases that average curves are of somewhat doubtful significance. We therefore selected 16 cases where parallax errors seemed minimal, when the bat did not start from the wall, and when the search phase was relatively uncomplicated by immediately previous manoeuvres. On averaging these for each bat, the mean pulse-to-pulse interval was found to increase rather smoothly to reach a plateau value at 70 cm. for *M. lucifugus* S, 55 cm. for B, and 90 cm. for the *M. keenii*. Parallax errors render these values too high by perhaps 20 or 25 per cent. but detection at half a metre would appear to have been common, and an occasional detection may well have occurred at one metre.

e. Measurements with Two Cameras

After these records had been analysed we made a further effort to determine the distance of detection more accurately by using two 16 mm. motion picture cameras to photograph many *Drosophila* catches by two *M. lucifugus*, TL and TR, which were two of the better catchers under our experimental conditions. Both were Auricon sound-on-film cameras operated by synchronous motors at 24 frames per second. Each received the same input of rectified pulses from the microphone, and each photographed a clock with minute and second hands. To aid in correlating the two films, the field of each camera also included a clock hand that revolved once per second and an oscilloscope with the horizontal sweep set at 20 per second. Careful study of several hundred feet of paired films permitted the selection of 11 clear cases in which a *Drosophila* catch could be unequivocally located on both films, i.e. both bat and fly were depicted throughout search, approach, and terminal phases, and there was no doubt concerning the matching of individual frames of the two films. In each pair of pictures the bat or fly appeared against the different spots on the grid on the opposite wall, and lines could be drawn on a

floor plan of the room from the positions of the camera to these projections on the wall. The intersections of these lines located bat or fly within one or two inches. The same accuracy could equally well have been achieved in the vertical position, but in all eleven cases the bat and fly were either flying almost horizontally, or were diving or climbing too slowly to introduce appreciable errors when vertical movements were ignored.

For these eleven cases we thus obtained fairly precise plots of the positions of bat and fly every 24th of a second, and from these we could measure the distance from bat to fly. We could then plot the pulse-to-pulse interval on the same time base as the distance separating the bat from its target. Three samples of the resulting combined graphs are shown in Figs. 2, 3, and 4; these are representative, although they include more clear-cut shifts from search to approach phase than some of the other cases. All eleven cases permit fairly accurate estimates of the distance of detection, however, and these distances are listed in Table II, along with the angular position of the fly relative to the bat's flight path at the time when the search phase ended and the approach phase began. It is quite evident that these more accurate measurements confirm the tentative conclusion reached above that detection commonly occurred at about half a metre. The shortest measured distance of detection was 23 and the longest 83 cm.

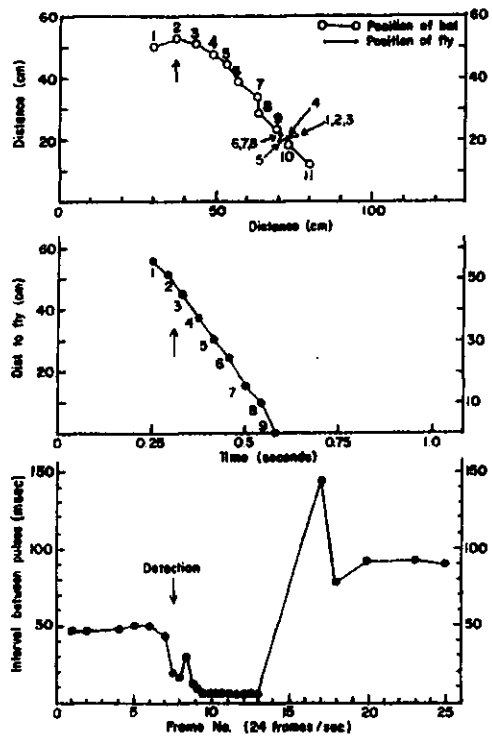

Fig. 2. Positions of bat and fruit fly and the pattern of orientation sounds during the catch. The upper graph shows a floor plan of part of the flight room some distance from the walls in which the zero point is arbitrarily selected to show the bat's position, and that of the fly, as determined by cross bearings from two motion picture cameras. The middle graph shows the distance separating bat and fly, and the numbers on the upper graph and middle graphs refer to frames of the film. The lower graph shows, on the same time base as the middle graph, the intervals between pulses. The arrow indicates the assumed point of detection. As in Fig. 1 the buzz contained more pulses than can be depicted individually.

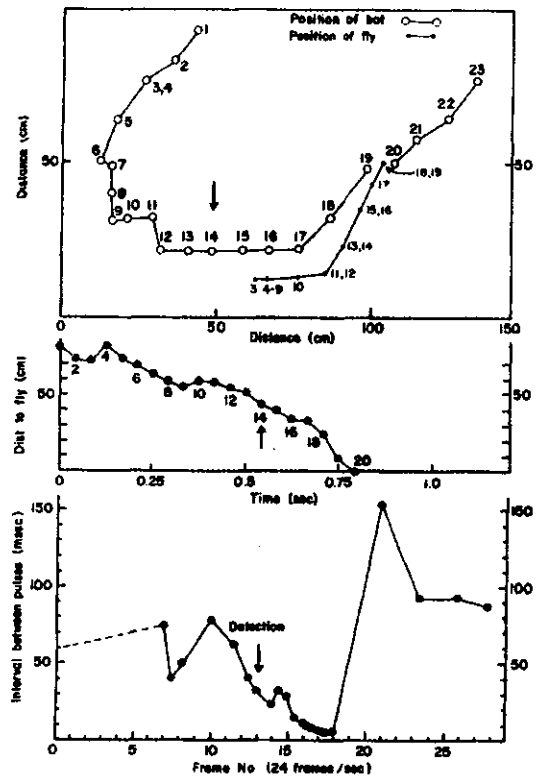

Fig. 3. Positions of bat and fly, together with the pattern of sound emission in another catch manoeuvre. For details see legend for Fig. 2.

The motion pictures suggest that the bat turns towards its prey within a small fraction of a second after detection. It is also of considerable interest to note that many detections seem to have occurred at considerable distances even though the fly was not directly in front of the bat.

Table II. Distances and Directions at which *Drosophila* were Detected by Two *Myotis lucifugus*. Detection is assumed to occur when the pulse-to-pulse interval first drops below the level characteristic of the search phase. Direction is given relative to the bat's flight direction at the time of detection.

Catch no.	Bat	Dist. of detection (cm.)	Direction of fly at time of detection (degrees)	Remarks
1	TR	62	15°L.	Bat had just turned away from wall, fly moving toward bat, detection may have occurred earlier, perhaps at 80 c.m.
2	TL	52	5°R.	Bat approaching at 90°
3	TL	68	60°R.	Approach from behind fly
4	TR	23	50°L.	Oblique approach from behind fly
5	TR	34	15°L.	Fly almost stationary, straight approach
6	TR	45	45°R.	Fly stationary, straight approach (Fig. 2)
7	TR	27	40°R.	Oblique approach from behind fly
8	TL	42	15°L.	Approach from behind fly (Fig. 3)
9	TL	83	35°R.	Fly approaching bat, detection may have been at about 90 cm.
10	TL	56	35°R.	Approaching at about 90° (Fig. 4)
11	TR	40	70°R.	Bat out of field of one camera during part of approach phase
Average distance of detection		48		

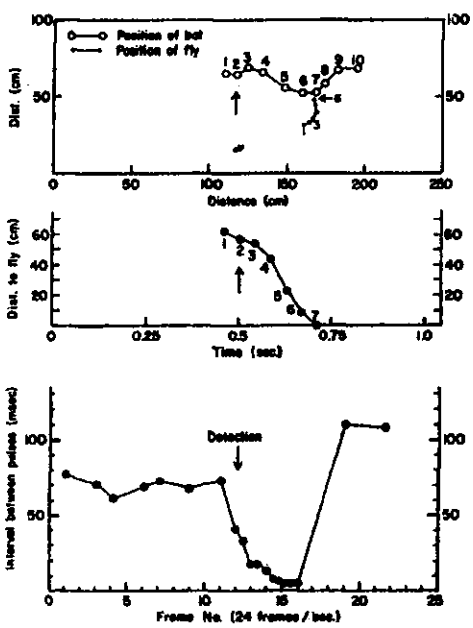

Fig. 4. Positions of bat and fly together with the pattern of sound emission in a third catch manoeuvre. For details see legend for Fig. 2.

Indeed there is little if any correlation between the angular position of the fly at the time of detection and the distance at which detection occurred. Since these bats usually seem to face in approximately the direction they are flying, at least in the search phase, this indicates that *Drosophila* can be detected from anywhere within a cone of perhaps 120°.

4. Selective Jamming Experiments with Low and High Frequency Noise

The evidence presented above suffices to show that small insects such as mosquitos and fruit flies are detected rapidly and at distances of about 50 cm. But even when taken together with the marked adaptation of the tempo of orientation sounds, this evidence does not prove conclusively that the insects are detected by means of echoes. It would still be logically possible to postulate that detection was achieved by hearing sounds of the insects' wing-beats. *Drosophila* were selected for the above experiments partly because their flight is very quiet, so that detection by passive listening would be more difficult than in the case of mosquitos or many of the larger flies and beetles

Furthermore, Williams & Galambos (1950) have measured the wave form of the flight sounds from a single *Drosophila funebris* beating its wings while attached to a small wire. The fundamental frequency under these conditions was about 180 cycles per second, and while the waves were complex enough to demonstrate the presence of strong harmonics, there do not appear to be any above the 10th at appreciable amplitude. The sound of a single *Drosophila* is very faint; Williams & Galambos used a 640AA condenser microphone at 1·5 cm. from the fly but did not estimate the absolute sound pressure level.

We have measured the sounds emitted by *Drosophila melanogaster* when 20-30 at a time were placed in a small glass bottle (about 50 millilitres) covered at the top by gauze. When the flies were strongly agitated and buzzing vigorously the r.m.s. sound pressure level was 25-30 decibels above 0·0002 dyne/cm.^{2} at 15 cm. from the gauze covered end of the bottle. This sound level, measured with a calibrated 640AA microphone, must have been considerably higher than that from a single fly in flight at 50 cm. from a hunting bat; probably the level reaching the bat's ears at this typical distance of detection would have been below 20 decibels. The observed frequency was between 500 and 800 cycles/second which is considerably higher than that measured by Williams & Galambos. It seems probable that resonance phenomena associated with the bottle may have accounted for this difference, but for present purposes the important point is that 20 or 30 flies, aided by the bottle's resonance, produced such a low sound pressure level.

If these bats detect fruit flies by hearing their flight sounds, it should be possible to mask these low frequency and low intensity sounds from the fly without employing a masking noise of high enough frequency to interfere with the bat's echolocation. Our best microphones when used together with an ultrasonic spectrum analyser could detect no ultrasonic components in the flight sounds of *Drosophila*, even though adequate to reveal sounds as faint as 25-30 decibels, above 0·0002 dyne/cm.^{2} We can thus exclude the posibility that *Drosophila* are detected by hearing ultrasonic components of their flight sounds, unless these components lie below this level of intensity. For selective masking experiments we therefore set up a simple apparatus to generate noise over the frequency band from about 50-15,000 cycles/second. It consisted of a Grason-Stadler noise generator, a 20-watt high fidelity audio amplifier, and a combination of a standard 8-inch dynamic loudspeaker with an Electrovoice driver loudspeaker for the higher audible frequencies. This system filled the small flight room with a noise so loud that we had almost to shout when we wished to communicate. For comparison with this loud low frequency noise a rather weak thermal noise in the bat's frequency range was also used for certain experiments. It was generated by a General Radio type 1390A noise generator, a broad band amplifier which delivered into four Isophon electrostatic loudspeakers about 50 volts r.m.s. These loudspeakers gave a declining output above about 25 kilocycles/second, but still emit an appreciable signal to 55 kilocycles, and a weaker signal at least over much of the range from 55 to 100 kilocycles. For some experiments electronic filters were used to restrict the frequency band of the low or high frequency noise. The low frequency noise sounded roughly equal in intensity throughout the small flight room used for observations of *Drosophila* catching, but the intensity of the high frequency noise undoubtedly varied widely from point to point, and it was not measured. Measurements with a calibrated 640AA microphone placed the overall sound pressure level of the 100-5,000 cycles/second band of the low frequency noise at +87 decibels re 0·0002 dyne/cm.^{2} Its spectrum level did not vary greatly from 100-8,000 cycles/second as judged by ear and by measuring the level passed by a narrow band filter moved gradually across the frequency range from 100-10,000 cycles/second. The noise level was thus approximately 50 decibels per cycle bandwidth or 20-30 decibels more than the *overall* sound level of several *Drosophila* flying inside a small bottle.

When *Myotis lucifugus* which has previously been good fruit fly catchers were exposed to these two noises the results were consistent and unequivocal. The low frequency noise had no noticeable effect; the bats continued to catch flies as rapidly in this noise as in the quiet. The high frequency noise, however, produced a marked decrease in the number of pursuit manoeuvres, the number of buzzes, and in the rate at which the bats increased weight while hunting. They did not stop hunting altogether, but usually made many fewer attempts to catch fruit flies, and then landed. If the high frequency noise was switched off they would often resume

Table III. Rates at which *Myotis lucifugus* Captured *Drosophila* in Low and High Frequency Noise. Rates of capture are based on the measured rate of weight gain divided by an assumed average weight of 2 mg. for the fruit flies, and hence are minima. Bat TR was used July 2-27, 1959, and bat TL August 23-24.

Date and time	Conditions of noise and light		Interval between weighings (min.)	Rate of insect capture (flies per min.)	Remarks
July 2	0·05—5 kc.	dark	10	7·5	
July 18	0·05—5 kc.	light	10·5	11	
July 19	0·05—5 kc.	light	15	14·8	
July 20	0·05—5 kc.	light	12·8	4·7	
July 22 2:06	Quiet	light	12	6	
Jiuy 22 2:22	0·05—15 kc.	light	6·7	14·2	
July 27 1:40	0·1—15 kc.	light	3	13·5	
July 27 1:51	20—100 kc.	dark	3	—	Lost 1·7 mg./min.
July 27 2:00	Quiet	dark	3	11·7	
July 27 2:12	40—100 kc.	dark	3	1	
July 27 2:19	0·1—15 kc.	dark	3	3·8	Flies scarce
July 27 2:27	Quiet	dark	3	3·4	Flies scarce
Aug.23 2:32	0·1—15 kc.	light	6	13·7	
Aug.23 2:44	20—100 kc.	light	6	—	Lost 11 mg./min.
Aug.23 2:56	Quiet	light	6	9·3	
Aug.23 3:07	40—100 kc.	light	6	—	Lost 10 mg./min.
Aug.23 3:18	0·1—15 kc.	light	6	6·9	
Aug.23 3:31	Quiet	light	6	3·2	Bat tired
Aug.24 4:00	0·1—15 kc.	light	3	13·1	
Aug.24 4:13	20—100 kc.	light	4	—	Lost 29 mg./min.
Aug.24 4:24	Quiet	light	4	10·9	
Aug.24 4:32	40—100 kc.	light	4	—	Lost 7·5 mg./min.
Aug.24 4:41	0·1—15 kc.	light	4	10·1	
Aug.24 4:47	Quiet	light	4	7·8	Bat tired, flies scarce

hunting almost at once. Excluding measurements obtained when the bat appeared tired or when the flies were scarce, the average rate of *Drosophila* capture was 9·5 per minute in the quiet and 11 per minute in the low frequency noise. Nor was there any appreciable difference between experiments in the dark and in the light. The results of the several experiments summarized in Table III thus provide strong evidence against the possibliity that under these conditions fruit flies were intercepted by passive listening to their feeble flight sounds.

Discussion

When one watches a six-gramme bat chasing two-milligram fruit flies with dextrous, agile, and split-second manoeuvres, it is natural to wonder whether it obtains enough food from such tiny insects to equal metabolic energy required to catch them. Even the best catchers in our ex-

periments have required supplementary feeding of mealworms, but this was primarily because we could not keep enough fruit flies on the wing to furnish a normal night's catch. The metabolic rate of a flying bat has been estimated as roughly 100 calories per gramme per hour or 600 calories per hour for a six-gramme bat (Griffin, 1958). The caloric value of a *Drosophila* does not seem to have been measured, but a reasonable approximation is probably the value of 2241 calories per gramme listed by Trager (1953) for newly emerged adults of the fly *Ophyra cadaverina*. When our bats were catching ten fruit flies per minute (conservatively assumed to average 2 milligrams in weight) they were taking in food at a rate of roughly 2,700 calories per hour, provided the above assumptions are correct. Even though the supreme exertion of insect interception may require somewhat more than 600 calories per hour, the game does seem to be worth the candle.

Since *Myotis lucifugus* can detect echoes from *Drosophila* at 50 centimetres or more, it is appropriate to inquire what the intensity of such echoes would be. This will depend upon the sound level emitted by the bat, the frequency chosen for consideration, and the effective size of the fruit fly; but an approximate formula for the necessary computation is available (Griffin, 1958, p. 349). In the search phase the r.m.s. intensity of the outgoing pulse at 100 millimetres from the mouth of a *Myotis lucifugus* is about 100 decibels above the standard reference level of 0·0002 dyne/cm^2. This is the maximum value when the pulse is at its highest amplitude, and this peak amplitude occurs at roughly 60-70 kilocycles, so that the strongest component of the echo corresponds to a wave length of about 5 mm. For simplicity let us assume that the *Drosophila* returns an echo of 5 mm. sound waves equal to that from a rigid sphere of 2 mm. radius, a rough approximation but one that we cannot improve upon until actual target cross sections of insects for bat pulses are measured. Making the above assumptions one can compute that the echo from a *Drosophila* would have an r.m.s. value of about 30 decibels at a distance from bat to fly of 50 cm., approximately the distance by which our records show detection to have occurred. This calculation assumes that the inverse square law applies to both the emitted sound beyond 10 cm. from the bat's mouth, and to the echo from the fruit fly. It also takes into account the 2½ decibels of atmospheric attenuation at 68 kilocycles over a round trip distance of 1 metre. If the fly were detected at one metre, rather than 50 cm. the echo would be reduced by sixteen fold, or by about 12 decibels. This, together with another 2½ decibels of atmospheric attentuation, would reduce the echo to 15 decibels, only slightly above the human auditory threshold at those frequencies where it is lowest.

Detection by bats of an echo having an intensity between 15 and 20 decibels appears plausible, except that the human auditory threshold of sound intensity rises sharply as the duration of a tone is decreased below about 200 milliseconds (Stevens, *et al.*, 1951, pp. 1,020-1022). These data suggest that a 1-millisecond pulse might have a threshold 100 times, or about 20 decibels higher than one lasting 200 milliseconds. Since 0 decibels is approximately the human threshold under favourable conditions, it does not strain credulity to postulate that a bat could detect one-millisecond pulses at 15 decibels especially since there is good reason to believe that the auditory brains of bats are highly specialised for the detection of short pulses of high frequency sound (Grinnell & Griffin, 1959).

Another important question concerns the choice of 68 kilocycles, or a wavelength of 5 mm. as the most easily detectible portion of the echo. Other factors being equal, higher frequencies or shoter wavelengths would return somewhat stronger echoes, but higher frequencies are not present at as high intensities in the emitted pulse, they suffer more atmospheric attenuation, and furthermore in the case of spheres with a 2 mm. radius the increase in echo is not very rapid between 68 and 120 kilocycles, the highest observed frequency in *Myotis* pulses. Lower frequencies are actually used during the interception phase, down to about 25 kilocycles in some cases. But of course the distance is then much shorter, and to a first approximation the echo intensity varies inversely as the fourth power of the distance. While the echo of a 25 kilocycles component would be weaker than that of 68 kilocycles by a factor of about six, the shorter distance would more than offset this.

These echoes which the bat seems to detect at a level of about 30 decibels may come from any direction within a cone of roughly 120°. Nor is mere detection enough for the hungry bat; it must localise the direction from which the echo is coming and execute an appropriate turn within a fraction of a second. The probing pulse of sound emitted by a *Myotis* has a rather broad angular spread, although the higher frequencies at the start of the pulse are more sharply con-

centrated into the forward direction than the lower frequencies at the end. It is possible that in each of the cases shown in Table II where the angular position of the fruit fly was far removed from the flight path of the bat the animal happened to have its head turned in that particular direction at the moment of detection. But photographs of flying *Myotis* indicate that during the search phase the head is ordinarily pointed in the direction of flight, so that it seems more likely that echoes can be detected from anywhere within a fairly wide zone in front of the animal. The initial localization may not be precise, and the need for more accurate bearings may well explain the shortening of pulse-to-pulse interval during the approach phase. But an eventual understanding of the physiological mechanisms of echolocation must include not only the detection of insect echoes at a level of roughly 30 decibels, but their almost instantaneous localization as well.

Finally we should compare the difficulty of jamming the echolocation of small wires (Griffin & Grinnell, 1958) with the cessation of insect hunting in high frequency noise. It should be borne in mind that in the jamming experiments collisions with wires were presumably unpleasant enough to motivate their detection. But we have no way of ascertaining how strongly our bats were motivated to continue hunting when the noise was switched on. Insect hunting has been elicited only with difficulty, even in the quiet, and it is not surprising that high frequency noise would discourage it. In our jamming experiments with wires the bats preferred not to fly and they tried to avoid the difficult task of echolocating small wires in the noise. While we could make some of them do this by forcing them to fly in an array of wires, we have no comparable way of forcing bats to attempt insect catching in the noise. Furthermore, wires are larger in one dimension and are stationary, rather than moving targets, Bats often slow down on approaching wires, but insects are intercepted at flight speeds of at least one metre per second.

It is remarkable that our very loud low frequency noise did not reduce the rate of insect catching at all, and that the good catchers, TR and TL, caught approximately as many fruit flies in this noise as in the quiet. In human masking experiments low frequencies are found to mask higher tones more easily than the reverse, but the auditory system of a *Myotis* is clearly able to make sharp frequency discriminations (Grinnell & Griffin, 1959), and it must be able to distinguish 30 decibel echoes at 60 or 70 kilocycles from a noise covering the band from 0·1 to 10 or 15 kilocycles at a level of about 50 decibels per cycle bandwidth.

Another question might be phrased as follows: since bats can apparently hear echoes from wires at a signal-to-noise ratio of −35 decibels (Griffin & Grinnell, 1958), why not assume that they can also hear *Drosophila* wing beats 30 decibels below a 50 decibel per cycle bandwidth noise at lower frequencies? This question leads directly into the problem of critical bands for the hearing of bats, a problem discussed elsewhere (Griffin, 1958, pp. 373-375). But regardless of the uncertainties surrounding this problem, it is difficult to believe that bat hearing or *Drosophila* flight sounds could have so narrow a bandwidth as one cycle per second. Hence the noise effectively competing with the flight sounds must be greater than 50 decibels. Even if we assume that the overall level of the *Drosophila* flight sounds (about 20 db. re 0·0002 dyne/cm^2) is spread over as narrow a bandwidth as 10 cycles/second, the competing noise would have a level of 60 decibels or 40 decibels above the sound level from the fly.

It should be reiterated that we are not suggesting that all detection of insect prey by all bats under all conditions is based upon echolocation. The observations of Moehres (1950), Kolb (1959) and others are sufficient to demonstrate passive detection of relatively noisy insects, and we have also observed bats suddenly respond to a buzzing blow fly and turn towards it from a distance of several feet. Quite clearly both active echolocation and passive detection are important in the feeding behaviour of bats under natural conditions. We have considered only the former, because of its intrinsic interest, and because its importance had not been fully recognized heretofore.

Further experiments now in progress seem likely to throw additional light on the intricate and rapid flight manoeuvres executed during insect pursuit and interception, the ways in which the various membranes are employed, and the accuracy of localization of the insects. These studies will be reported elsewhere, together with a consideration of the information processing involved in the tracking of small moving targets.

Summary

1. Bats of the genus *Myotis* (*M. lucifugus*, *M. subulatus leibii* and *M. keenii septentrionalis*) have been studied while pursuing and capturing

small insects under laboratory conditions. It is apparently important to provide fairly large numbers of such insects in order to elicit insect catching behaviour indoors.

2. Insect catches are individually directed pursuit manoeuvres; each insect is detected, located, and intercepted in flight within about half a second.

3. Certain individual bats caught mosquitos (*Culex quinquefaciatus*) and fruit flies (*Drosophila robusta* and *D. melanogaster*) at remarkably high rates which could be measured conservatively by the gain in weight of the bat. Sometimes a bat would average as many as 10 mosquitos or 14 fruit flies per minute during a period of several minutes. In four cases motion pictures showed two separate *Drosophila* catches within half a second.

4. The orientation sounds of the hunting bat are adjusted in a manner that seems appropriate for the echolocation of single insects one at a time. There is a *search phase* before the occurrence of any apparent reaction to the insect. In this phase the frequency drops from about 100 to 50 kilocycles during each pulse of sound, and the pulses are emitted by *M. lucifugus* at intervals of 50 to 100 milliseconds.

5. When an insect is detected the search phase gives way to an *approach phase* characterized by a progressive shortening of the pulse-to-pulse interval and, if necessary, a sharp turn towards the insect. In this phase the pulse duration may shorten somewhat, but the frequencies remain approximately the same as in the search phase or drop slightly.

6. When the bat is within a few centimetres of the insect there is a *terminal phase* in which the pulse duration and interval between pulses shorten to about 0·5 millisecond and 5 or 6 milliseconds respectively. Contrary to a conclusion reached earlier on the basis of much less adequate data (Griffin, 1953), the frequency *drops* in the terminal phase, sometimes to 25 or 30 kilocycles. This is the buzz, which also occurs in many cases when the bat is dodging wires or landing.

7. The distance from the insect at which detection occurs can be judged by the shift from search to approach patterns. This distance of detection is commonly about 50 cm. for *Drosophila*, and it occasionally may be as much as a metre with fruit flies or mosquitos.

8. Two *M. lucifugus* which had become adept at catching *Drosophila* in the laboratory were exposed to broad band thermal noise either at low frequencies (0·1-15 kilocycles) or high (20-100 kilocycles). The low frequency noise had an approximately uniform spectrum level of about 50 decibels per cycle band width (re 0·0002 dyne/cm^2) from 0·1 to 8 kilocycles. It was thus very loud compared to the flight sounds of *Drosophila* which have a fundamental frequency of a few hundred cycles/second and a maximum sound pressure level of 20-25 decibels at the distances of detection by these bats. The high frequency noise was of low and varying intensity, but it discouraged or prevented insect catching. The low frequency noise, on the other hand, had no effect on insect catching; the bats gained weight in this noise (and in the dark) just as rapidly as in the quiet. Although bats sometimes detect insect prey by passive listening to sounds emanating from the insects themselves, these experiments appear to us to establish conclusively that small and relatively silent insects are often detected by echolocation.

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HUMAN EASTERN EQUINE ENCEPHALITIS IN MASSACHUSETTS: PREDICTIVE INDICATORS FROM MOSQUITOES COLLECTED AT 10 LONG-TERM TRAP SITES, 1979–2004

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Abstract. Human eastern equine encephalitis (EEE) is a life-threatening mosquito-borne disease. To determine whether mosquito abundance and EEE virus infection rates are associated with human EEE disease, we evaluated retrospectively a total of 592,637 mosquitoes and onset dates for 20 confirmed human cases over 26 years in Massachusetts. Annual *Culiseta melanura* populations at 10 defined sites decreased over the study period ($P = 0.002$). Weekly infection rates and number of infected *Culiseta melanura* captured per trap night were positively associated EEE cases ($P = 0.023$ and $P < 0.001$, respectively), whereas abundance was not ($P = 0.077$). The infection rate for *Culiseta melanura* of 0.39 per 1,000 tested mosquitoes identified human cases with a sensitivity of 0.87, a specificity of 0.82, a positive predictive value of 0.14, and a negative predictive value of 0.995. Timely mosquito testing and infection rate calculation are critical for disease risk estimation and outbreak control efforts.

INTRODUCTION

Human eastern equine encephalitis (EEE) is relatively rare (4.92 cases per year in the United States from 1964 to 2000), but the disease produces significant morbidity and mortality (31–74%) and profound neurologic sequelae in 55–96% of survivors.[1–5] Additionally, human EEE outbreaks have caused significant public concern, probably because of their intensity and infrequency.[5] Considering its serious consequences and social and economic impact, EEE is of public health importance.[5,6] Thus, accurate human risk estimation and outbreak prevention measures are needed.

EEE is a mosquito-borne disease caused by an alphavirus.[5] The primary hosts are wild birds and ornithophilic mosquitoes, *Culiseta melanura,* the enzootic vectors in the United States.[7,8] Infection among humans and horses is most likely incidental and occurs when the virus circulates widely and expands from the enzootic cycle.[9,10] Bridging vectors are believed to be in the genera *Aedes, Coquillettidia, and Culex*.[11–13] Monitoring mosquito abundance and EEE virus infection rates may offer useful estimation of human disease risk.[14]

The first human EEE outbreak was recognized in Massachusetts in 1938, and 34 cases with 25 fatalities were recorded.[15,16]

One case occurred the following year, but subsequent human disease occurred irregularly, and outbreaks often lasted > 1 year: for example, in 1955 and 1956 (16 cases); from 1970 to 1974 (7 cases); and from 1982 to 1984 (10 cases).[17–21] In the 1990s, three cases occurred in 1990 and one case each in 1992, 1995, and 1997. After single cases in 2000 and 2001, in 2004, the state experienced an EEE outbreak with four human cases (two fatalities) and seven equine cases. The overall mortality rate through 2004 was 50.6%. Based on historical trends, 2004 may be considered as the beginning of another outbreak. For this reason, timely human risk estimation is desirable during the next several years. The Massachusetts State Laboratory Institute has monitored entomologic and ecologic conditions and human EEE cases, but quantitative analysis, which evaluates the association of mosquito indicators with human disease, has been limited.

We carried out these analyses to test the hypothesis that the abundance and EEE virus infection rates among mosquitoes can quantitatively estimate the likelihood of human EEE disease. In particular, we described the annual populations of given mosquito species and provided the weekly mosquito density and EEE virus infection rates. Using these findings, we tested the association between these mosquito indicator values and human cases.

MATERIALS AND METHODS

Study sites. General descriptions of the study sites, their locations, and the climate have been reported previously.[20] In brief, the area chosen for this study was southeastern Massachusetts, specifically Bristol (five sites), Plymouth (four sites), and Norfolk (one site) counties, where the majority of human and horse cases have occurred. All sites are located in large areas of forested wetlands, densely covered by white cedar/red maple swamps, and were chosen primarily to collect *Cs. melanura*.

Sampling procedure. Battery-operated standard CDC miniature light traps without CO_2 were used to sample adult mosquito populations from late May or early June to late September or early October. Collection usually ended after the first hard, extensive frost occurred. A pair of traps hung ~1.5 m above the ground was placed far enough apart to avoid competition.[20,21] Traps were set once or twice weekly and were picked up the following morning. Collections were stored on dry ice and transported to the State Laboratory Institute (SLI). Female mosquitoes were sorted on the basis of date, site, trap number, and species and processed for virus isolation. Weekly mosquito abundance was described as mean number captured per trap per night during a week. A week was defined from Saturday to Friday, with the first week ending on the first Friday of the year. In this study, we analyzed data from 1979 to 2004, collected using CDC light traps set by the SLI staff at 10 long-term sites only. Mosquitoes collected in other types of traps, at other sites, and by regional Mosquito Control Districts were excluded from the analysis.

Virus identification. Mosquitoes were sorted by species and sex, pooled into groups of 50 or less, and ground in diluent.

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Plaque assay, on primary chick embryo tissue culture through 1996, and subsequently on Vero cells or BHK-2 cells, was performed on all supernatants of pools of female mosquitoes and followed by immunofluorescence assay for identification.[22,23] Virus isolations were expressed as minimum infection rates (MIRs, number of virus positive pools per 1,000 mosquitoes tested).[14] This standard computation for MIR ignores the possibility that multiple mosquitoes may be infected in a pool.

Case definitions. Case surveillance and definitions were consistent with CDC recommendations. SLI identified 22 human cases of EEE during the study period. Laboratory confirmation was made by detection of EEE specific IgM antibody in the cerebrospinal fluid (CSF), virus isolation from patients' brains or CSF, or serum antibody titers increasing 4-fold or more from acute to convalescent phase or converting from IgM to IgG using IgM- and IgG-specific enzyme immunoassays and plaque reduction assays.[22,24,25] Two cases were excluded from the analysis, because epidemiologic evidence supports that they were exposed in New Jersey or in Hampden County, Massachusetts, > 100 km from the study sites. Illness onset dates for the remaining 20 cases were included.

Data analysis. A simple linear regression was used to examine whether the mosquito population and MIR have changed over time. Poisson regression was used to study whether the risk of human EEE disease was associated with mosquito indicators. We compared mosquito indicator values in years with cases and those without using Wilcoxon rank sum test. Categorical data were compared by Fisher exact test. A receiver operator characteristic curve and the area under the curve were used to estimate whether mosquito indicators classify human disease and to compare different indicators. All statistical analyses were performed using Intercooled Stata 8.0 (Stata Corp., College Station, TX). Statistical significance between groups was determined with two-tailed tests, and values of $P < 0.05$ were considered significant.

RESULTS

Data for a total of 612,265 mosquitoes representing seven genera and 23 species were stored in a database from June 1979 through October 2004, and 4 species were systematically differentiated (Table 1). Although *Cs. melanura* was the largest group throughout the study period (84.6%), *Coquillettidia perturbans, Ochlerotatus canadensis*, and *Aedes vexans* have been recorded as epizootic vector candidates in Massachusetts.[20,26,27]

Annual mosquito abundance and human cases. To determine the chronologic mosquito density trends, we compared annual mean abundance in each species. *Cs. melanura* significantly decreased during the study period ($P = 0.002$; Figure 1, top). Abundance of *Cs. melanura* dropped at all sites, and the results were statistically significant in 7of 10 sites. On the other hand, the density of *Cq. perturbans, Oc. canadensis,* and *Ae. vexans* did not decrease ($P = 0.30$, $P = 0.74$, and $P = 0.68$, respectively) but fluctuated (Figure 1, middle). Human cases clustered from 1982 to 1984, in 1990, and in 2004 and did not decrease over time ($P = 0.89$; Figure 1, bottom). *Cs. melanura* was abundant before and during the first two epidemics (from 1982 to 1984 and in 1990) but was less plentiful in 2004. Annual *Cs. melanura* collections are decreasing; however, the abundance does not correlate with the number of human cases.

Annual minimal infection rate among mosquitoes. To determine the chronologic EEE virus MIR trends among mos-

TABLE 1
Annual recorded mosquitoes by species captured in unbaited CDC light traps at 10 sites in Massachusetts, 1979–2004

Year	Date (first-last)	Traps	*Cs. melanura*	*Cq. perturbans*	*Oc. canadensis*	*Ae. vexans*
1979	Jun 4–Sep 18	424	28,190	1,532	3,767	485
1980	Jun 2–Sep 23	406	24,145	1,280	440	41
1981	Jun 14–Sep 15	342	5,128	314	284	28
1982	Jun 7–Sep 15	319	34,518	*	*	*
1983	May 16–Oct 11	485	19,399	1,105	123	20
1984	May 29–Oct 2	583	55,513	5,050	563	101
1985	May 30–Sep 28	571	23,367	3,516	393	112
1986	Jun 1–Sep 24	548	34,077	4,960	637	572
1987	May 11–Oct 6	464	9,096	1,173	134	48
1988	May 4–Sep 19	584	31,547	1,522	1,102	957
1989	May 9–Sep 25	482	38,951	1,267	2,383	1,585
1990	Jun 4–Sep 24	462	33,805	5,380	1,865	208
1991	May 20–Sep 30	613	19,410	5,042	222	96
1992	May 11–Oct 5	623	25,520	2,167	671	362
1993	May 17–Sep 27	626	12,207	1,614	581	13
1994	May 9–Sep 26	614	12,088	1,501	626	89
1995	May 8–Sep 25	640	16,128	1,515	208	40
1996	May 13–Sep 29	613	10,712	814	782	53
1997	May 12–Oct 5	619	11,551	2,090	733	11
1998	May 5–Oct 17	608	33,441	2,053	1,698	214
1999	May 31–Oct 4	377	6,425	973	62	24
2000	May 29–Sep 30	357	5,876	600	728	147
2001	May 29–Sep 18	316	13,399	813	285	217
2002	May 26–Sep 22	284	3,793	703	767	136
2003	May 28–Sep 13	259	4,413	560	691	102
2004	Jun 1–Oct 6	305	5,475	602	786	125
Total		12,524	518,174	48,146	20,531	5,786

* Not recorded.

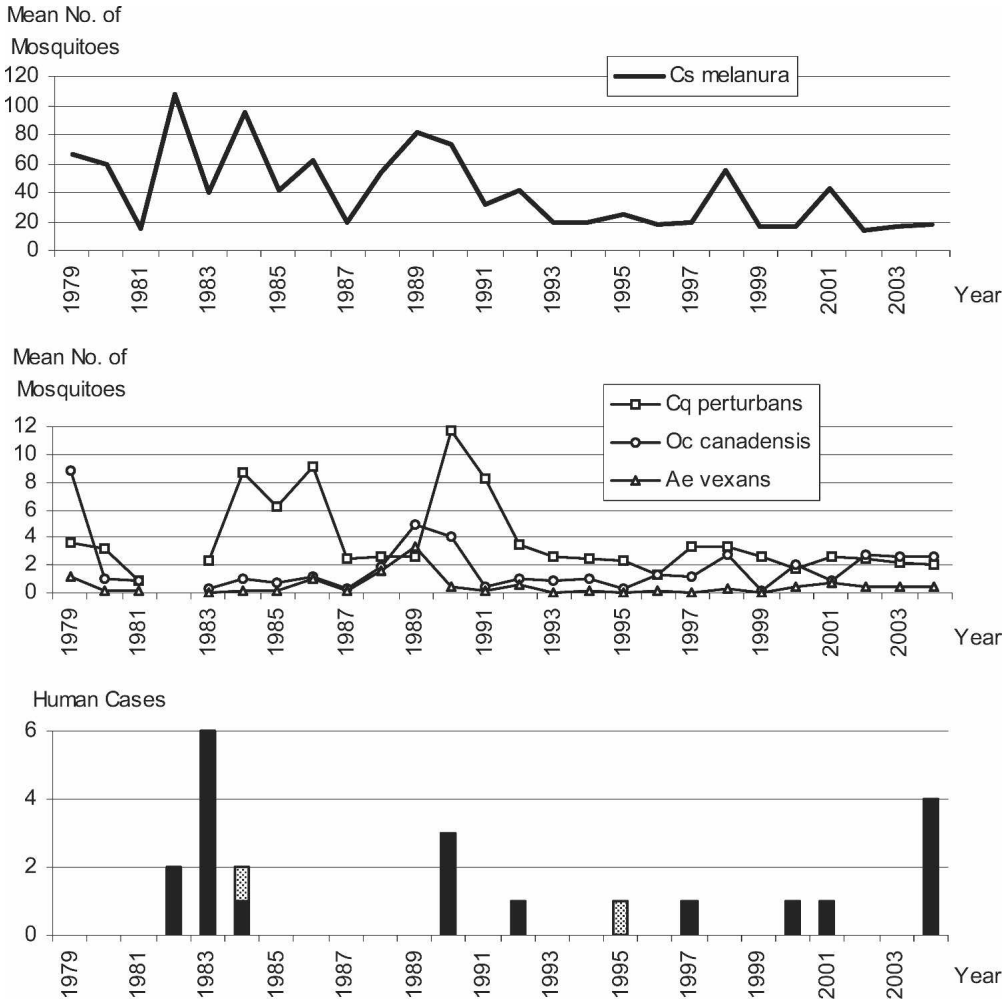

FIGURE 1. Mosquito abundance in unbaited CDC light traps at 10 long-term sites (top and middle) and human cases (bottom) in Massachusetts, 1979–2004. Numbers of mosquitoes were calculated as mean number of captured mosquitoes per trap per night during the entire season. Two cases presumably exposed to the virus outside of eastern Massachusetts are indicated as shaded bar (bottom). Cs, *Culiseta*; Cq, *Coquillettidia*; Oc, *Ochlerotatus*; Ae, *Aedes*.

quitoes, we compared annual MIRs (Table 2). In total, 19,362 mosquito pools were examined for virus, with 376 EEE recorded as positive and *Cs. melanura* accounting for the majority of positive pools (98.7%). Overall MIR for *Cs. melanura* was 0.71, and yearly MIR oscillated and has not decreased over time ($P = 0.21$). Overall MIR for *Cq. perturbans* (0.096) and *Oc. canadensis* (0.068) were significantly lower than that of *Cs. melanura* ($P < 0.001$ and $P = 0.001$, respectively). Annual EEE virus MIR for *Cs. melanura* varied. No significant associations were observed between annual MIR values and the number of human cases.

Weekly mosquito indicators and human cases. To estimate the relative association of EEE virus MIR and abundance of *Cs. melanura* on human disease, we plotted the seasonal distribution of mosquito indicators for 9 years with and 17 years without human cases. EEE virus–positive pools were found earlier in years with than in years without human cases (Figure 2, top). The former had greater MIRs than the latter throughout the season. The MIR differences were significant in weeks 29, 33, 34, 36, and 38 ($P = 0.048$, $P = 0.0046$, $P = 0.0066$, $P = 0.018$, and $P = 0.0013$, respectively). The MIRs were higher late in the season. Indeed, 7 of 9 human case years and 6 of 17 non-case years had their highest weekly MIRs during or after week 37. The difference in mean abundance between the two groups seems the greatest from July to August, but for each week, the values were not significantly different (Figure 2, middle). Human cases occur as *Cs. melanura* MIR and abundance increase (Figure 2, bottom).

Minimal infection rates in years with multiple cases. To elucidate the chronologic association between MIRs and human disease, we described weekly data in three different years with multiple cases (Figure 3). Human EEE cases tended to cluster in 1983 (first five patients occurred within 15 days) and 1990 (three patients within 8 days), but were scattered in 2004 when the onsets were at least 12 days apart. MIR levels at, or 1 or 2 weeks before, the onset dates were > 1.0 for all cases shown in the figure. The highest MIRs for the 10 long-term sites were 13.51, 7.49, and 12.05 in 1983, 1990, and 2004, respectively. Human case onset dates follow high MIR values during EEE outbreaks.

Mosquito indicators association with human cases. To quantify the association between mosquito indicators and human EEE disease, we compared weekly mosquito indicator values and the number of human cases in the same week.

TABLE 2

Tested and EEE virus-positive pools and EEE virus MIR for *Cs. melanura* captured in unbaited CDC light traps at 10 sites in Massachusetts, 1979–2004

Year	Tested	EEE (+)	MIR
1979	832	54	1.916
1980	793	5	0.207
1981	297	0	0
1982	826	49	1.42
1983	493	39	1.908
1984	1,388	20	0.36
1985	635	29	1.241
1986	88	1	0.029
1987	289	11	1.209
1988	773	4	0.127
1989	887	6	0.154
1990	786	74	2.189
1991	550	24	1.236
1992	683	7	0.274
1993	437	1	0.082
1994	433	0	0
1995	506	0	0
1996	404	0	0
1997	430	17	1.472
1998	785	6	0.179
1999	230	2	0.345
2000	221	6	1.021
2001	371	6	0.448
2002	170	0	0
2003	178	1	0.227
2004	428	9	1.182
Total	13,913	371	0.712

Coquillettidia perturbans and *Ochlerotatus canadensis* showed 4 and 1 EEE virus–positive pools among 2,476 and 2,136 tested pools, respectively.

EEE virus MIRs were significantly associated with human disease ($P = 0.023$), whereas abundance alone was not ($P = 0.077$). The third index, number of infected *Cs. melanura* per trap per night, which is the product of the former two indicators, showed the most significant association with human cases ($P < 0.001$). Abundance of *Cq. perturbans, Oc. canadensis,* and *Ae. vexans* were not associated with human EEE disease ($P = 0.26$, $P = 0.91$, and $P = 0.86$, respectively). Years with virus positive zoophilic mosquitoes were not associated with those with human diseases ($P = 0.45$). For practical use, we reported sensitivity and specificity for a variety of indicator values using the receiver operator characteristic curve and calculated the area under the curve (Figure 4). Area under the curve for MIR was 0.83 (95% confidence interval [CI]: 0.74, 0.93) and was significantly greater than that for abundance (0.63; 95% CI: 0.48, 0.78; $P = 0.019$). The greatest accuracy was obtained at an MIR of 0.39, which classifies human cases with a sensitivity of 0.87, a specificity of 0.82, a positive predictive value of 0.14, and a negative predictive value of 0.995. The area under the curve for the number of infected *Cs. melanura* was 0.83 (95% CI: 0.73, 0.92) and was not significantly different from that of the MIR ($P = 0.78$). The greatest accuracy was obtained with a value of 19.29 infected *Cs. melanura*, which detects human cases with a sensitivity of 0.87, a specificity of 0.81, a positive predictive value of 0.13, and a negative predictive value of 0.995.

DISCUSSION

Our study has three major findings. First, annual collections of *Cs. melanura* have decreased significantly at 10 trap sites since 1979. Second, weekly EEE virus MIR and the number of infected *Cs. melanura* per trap per night were associated with human disease, whereas abundance of this vector alone and that of other vectors were not. Finally, weekly EEE virus MIR and the abundance of infected *Cs. melanura* considered together were also able to estimate the likelihood of human EEE disease in the same week.

We found that the captured *Cs. melanura* in southeast Massachusetts have decreased. Possible explanations of our findings are as follows. First, the important wetland habitat for *Cs. melanura* might have decreased near collection sites. Although forest cover declined as a result of industrial, transportation, and housing development from 1971 to 1999,[28] the overall acreage of white cedar swamp has probably not decreased substantially in the past few decades (G. Motzkin, Harvard Forest, personal communication, 2005). Total white cedar wetland may be relatively stable, but individual swamp patches have been altered.[29] Second, in recently developed areas, illumination from street lights or houses may compete with light traps and cause reduced mosquito collections. SLI staff recently conducted field assessments that revealed extensive woodcut in a single site and construction of new houses, buildings, and a pump station not far from trap locations in at least 4 of 10 sites in the past few years (unpublished data). Although these assessments were limited, reduced collections of *Cs. melanura* were most likely caused by diminished habitat around the trap sites. Trap site conditions should be evaluated periodically to obtain sufficient mosquito vectors for analysis.

Weekly MIR differences between years with and without human cases combined with a significant association between mosquito indicators and human illness clearly showed that *Cs. melanura* plays an essential role in EEE virus transmission. Of the three mosquito indicators, the strongest association with human disease was observed with the number of infected *Cs. melanura.* Although previous reports identified that the abundance of this vector correlated with mammalian disease, we could not find a significant association as was observed with horses in New York.[30–32] We confirmed that vector abundance alone is not associated at a statistically significant level with human EEE disease.[5]

Unlike most reports, associations between zoophilic mosquito indicators and human disease were not found. Because these vectors have been thought to be responsible for mammalian infection in the state from a variety of evidence,[33,34] these results may well be caused in part by small numbers of captured mosquitoes and low infection rates. There are two possible approaches to clarify the roles of human biting vectors. First, trapping strategy could be changed to obtain more mosquitoes. As we previously reported, CO_2-unbaited CDC light traps do not collect large numbers of human biting species.[35] Other types of traps set in other habitats may collect enough vectors for meaningful calculation; however, how many zoophilic vectors need to be collected in terms of human disease prediction remains unresolved.[36] Another possibility is implementing more sensitive testing for virus detection. Findings in this report are based on the results from culture-based tests, but these conventional methods may miss mosquitoes with low viral titers or non-replicating virus.[37] Real-time detection polymerase chain reaction (RTD-PCR) may detect larger proportion of infected vectors. SLI now performs RTD-PCR for all mosquito samples. Attention

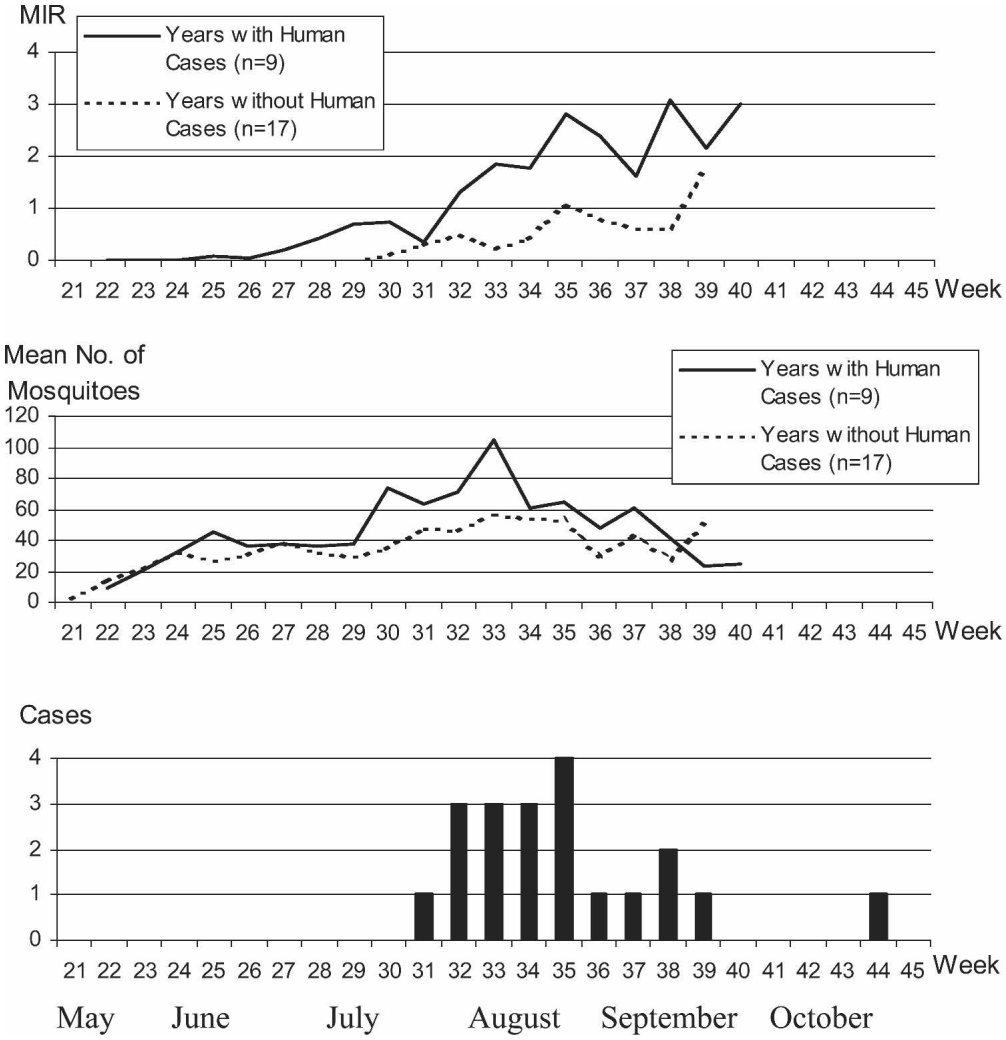

FIGURE 2. Mean weekly EEE virus MIR (top), mean weekly abundance (middle) of *Cs. melanura*, and human EEE disease onset dates (bottom) in Massachusetts, 1979–2004. MIR, minimum infection rate.

should be paid to interpretation and comparison to older data.

MIRs for *Cs. melanura* increased as its population decreased toward the end of the season. The same phenomenon was observed in Alabama and could be caused by blood meal preferences, host availability, and host–vector interaction change in late summer.[33,37–39] High MIRs do not seem to indicate a significant public health threat late in the season, because human-biting vectors become inactive in Massachusetts in late September,[27] and only one patient was reported with disease onset after the 39th week (Figure 1). Whether an increased MIR among *Cs. melanura* late in the season plays a role in over-wintering of EEE virus is not known.

The Massachusetts Department of Public Health has developed guidelines for a phased response to EEE virus surveillance data.[40] The guidelines include five risk categories that take into account prior and current year EEE virus activity in mosquitoes, equines, and humans. The risk of human outbreak probability ranges from level 1(remote) through level 5(critical). For example, level 1 requires no previous year or current year virus activity before July and recommends routine surveillance and measures to reduce mosquito breeding. On the other hand, level 5 requires multiple EEE virus isolations from mosquitoes, horses, or human cases and recommends stronger action such as active surveillance and increased adulticiding. This study offers quantitative basis for mosquito control policy in Massachusetts. This report presents sensitivity, specificity, positive predictive value, and negative predictive value to estimate human arthropod-borne disease risk on a weekly basis.

This work has some limitations. To eliminate bias and to have consistency in data collected and evaluated throughout the long study period, we excluded potentially useful data that was collected from supplemental trapping efforts. For instance, CO_2-baited light traps are set in response to increased virus activity in *Cs. melanura,* and additional surveillance sites are set in response to human or equine EEE cases. Extensive surveillance using gravid traps and CO_2-baited traps to collect *Culex* mosquitoes began in response to the arrival of West Nile virus (WNV) in New York City in 1999; these mosquitoes are tested for EEEV as well as WNV. In addition, for many years, regional Mosquito Control Districts have played a major role in mosquito surveillance for EEE and WNV in Massachusetts, with selected collections also

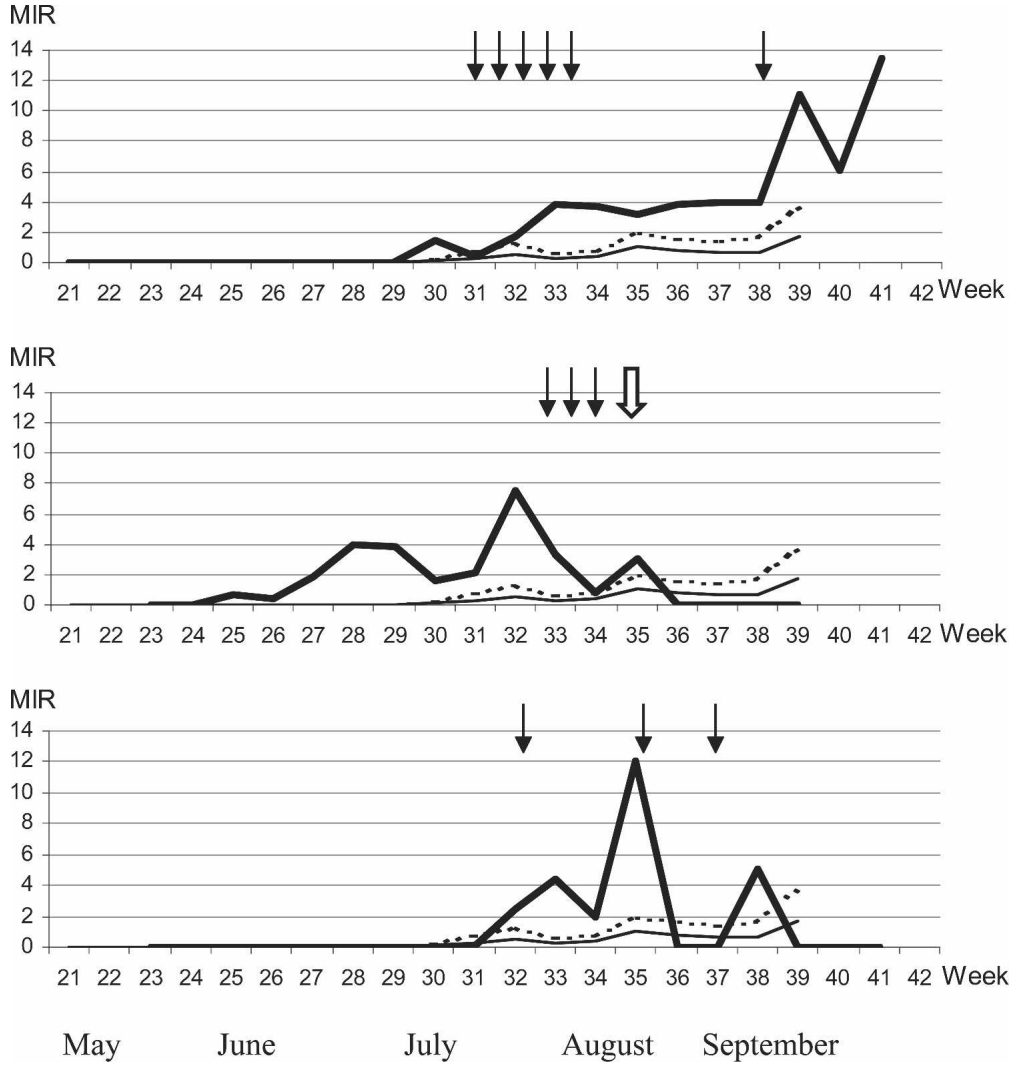

FIGURE 3. EEE virus MIR for *Cs. melanura* for each outbreak year (thick solid line), 1983 (top), 1990 (middle), and 2004 (bottom), in Massachusetts, 1979–2004. Thin solid and dotted lines indicate the mean and the upper 95% confidence interval of MIRs in 17 years without human cases. Arrows indicate human case onset dates and an open arrow represents aerial malathion application. A fourth case in the 44th week in 2004 is not shown. MIR, minimum infection rate.

tested at the SLI. Further analyses should include the supplemental data. Second, southeast Massachusetts is a large area, and we only estimate overall risk in this area. Site-specific data, however, is used to target areas for additional surveillance and possible responses. Third, during the time of this study, the lack of timeliness for confirming positive mosquito pools was a factor. Turnaround time was previously reported as 4 days for mosquito results from cell culture, but RTD-PCR has currently shortened this to as little as 24 hours from mosquito collection to report.[20] Considering that an EEE incubation period may be 5–7 days, public awareness should ideally be increased 1–2 weeks before the time of the highest risk.[41] Early awareness of the possibility of human EEE cases may also improve the timeliness of having appropriate clinical specimens tested at the SLI. Geographic Information System–based analyses for both mosquito and human data will allow us to improve the estimates of time-spatial risk assessment. For instance, data loggers to record site-specific weather conditions are now in place. Fourth, the positive predictive value of EEE virus MIR for *Cs. melanura* was low (0.14). This may be attributed to the low incidence of human EEE. A threshold value of an MIR of 0.39 for the long-term sites does have use, however, if one considers that values < 0.39 indicate a low risk of human disease; MIR values > 0.39 indicate the need for increased surveillance efforts, such as supplemental trapping and early notifications of the presence of virus to health care providers and the public. For practical purposes, an increasing or a sustained elevation in MIR in a focal area, taking into consideration all trapping, increases the level of response in those communities. Finally, mosquito indicators combined with other environmental and ecologic information, such as temperature and precipitation, wind trajectory, wild bird population and immunity, and EEE virus strains may offer more precise evaluation of human EEE disease risks.[19,20,42–46]

We provided a quantitative analysis of the association between mosquito indicators and human EEE disease risk. Our study is unique in reporting weekly population and EEE virus MIR for *Cs. melanura* captured by a single trap type at 10 fixed sites for 26 years with three human EEE disease out-

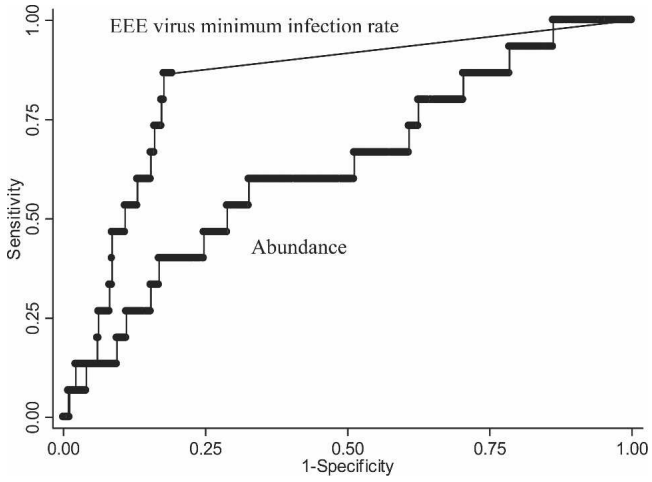

FIGURE 4. Receiver operating characteristic curve based on EEE virus MIR and abundance of *Cs. melanura* for human EEE cases.

breaks. EEEV MIR and the abundance of infected *Cs. melanura* are significantly associated with human EEE disease occurrence. Timeliness in the evaluation of suspect human cases for EEE plays a critical role in the assessment of public health risk. Indications of early season MIR values increasing above mean values must trigger increased active surveillance and fast-tracking of suspect human specimens for EEE virus and antibody testing. To prevent future outbreaks, it is crucial to conduct timely testing and MIR calculations and to use the data to inform and, if need be, alert Mosquito Control Districts, health care providers, and the public.

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Host Selection by *Culex pipiens* Mosquitoes and West Nile Virus Amplification

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Abstract. Recent field studies have suggested that the dynamics of West Nile virus (WNV) transmission are influenced strongly by a few key super spreader bird species that function both as primary blood hosts of the vector mosquitoes (in particular *Culex pipiens*) and as reservoir-competent virus hosts. It has been hypothesized that human cases result from a shift in mosquito feeding from these key bird species to humans after abundance of the key birds species decreases. To test this paradigm, we performed a mosquito blood meal analysis integrating host-feeding patterns of *Cx. pipiens*, the principal vector of WNV in the eastern United States north of the latitude 36°N and other mosquito species with robust measures of host availability, to determine host selection in a WNV-endemic area of suburban Chicago, Illinois, during 2005–2007. Results showed that *Cx. pipiens* fed predominantly (83%) on birds with a high diversity of species used as hosts (25 species). American robins (*Turdus migratorius*) were marginally overused and several species were underused on the basis of relative abundance measures, including the common grackle (*Quiscalus quiscula*), house sparrow (*Passer domesticus*), and European starling (*Sturnus vulgaris*). *Culex pipiens* also fed substantially on mammals (19%; 7 species with humans representing 16%). West Nile virus transmission intensified in July of both years at times when American robins were heavily fed upon, and then decreased when robin abundance decreased, after which other birds species were selected as hosts. There was no shift in feeding from birds to mammals coincident with emergence of human cases. Rather, bird feeding predominated when the onset of the human cases occurred. Measures of host abundance and competence and *Cx. pipiens* feeding preference were combined to estimate the amplification fractions of the different bird species. Predictions were that approximately 66% of WNV-infectious *Cx. pipiens* became infected from feeding on just a few species of birds, including American robins (35%), blue jays (17%, *Cyanocitta cristata*), and house finches (15%, *Carpodacus mexicanus*).

INTRODUCTION

In many parts of North America, mosquitoes from the *Culex pipiens* complex transmit West Nile virus (WNV) among individuals comprising diverse bird communities in a variety of landscapes.[1,2] West Nile virus has had local and regional impacts on bird populations,[3–5] yet just a few bird species, capable of being infected with WNV and then becoming infectious (competent hosts), may be responsible for most WNV maintenance and amplification.[6,7] These so-called super-spreader bird species, such as American robin (*Turdus migratorius*), are typically widespread, but are often not the dominant species in a community. The ornithophilic *Cx. pipiens* mosquito may demonstrate a preference for these super-spreader bird species. When *Culex* spp. feeding patterns are analyzed temporally, several studies have identified a shift in feeding from birds to mammals, which may enhance human epidemics.[8–10]

The contribution of a bird species to West Nile virus transmission depends on its host competence, which is a function of the magnitude and duration of viremia,[1,11,12] host-contact rates,[13,14] and survival rates. Host-contact rates are a function of vector feeding preferences[15] and relative abundance of susceptible hosts. Bird species with high reservoir competence with potential importance for transmission, such as American crow (*Corvus brachyrhynchos*[11]), are now understood to be less important, as shown by the observation that WNV transmission continues even where crow densities have been reduced[4] and because crows do not appear to be major hosts for *Culex* spp. mosquitoes.[16] Extensive serosurveys of avian communities have documented the presence of antibodies to WNV to identify spatial and temporal patterns of transmission.[17–23] However, serologic studies are limited because they quantify exposure rates only within the surviving fraction of the population that can be captured.[24] Such studies offer only limited insight into the actual contribution of different bird species to transmission. Identifying the role of different species in transmission through the integration of reservoir competence and mosquito feeding preferences has only been evaluated in the mid-Atlantic United States[6] and in Memphis, Tennessee.[7]

Mosquito host selection has been measured using forage ratios,[25] human blood index,[26] feeding index,[15] and feeding preference[6] but studies using these indices rarely incorporate fine-scale surveys of host availability. Host availability is a function of ecologic, biologic, and behavioral factors that influence the probability of a host being exposed to a mosquito.[27] Ecologic factors important for host availability include the night-time roost size, location, and height of a bird species. Biologic factors, such as host body mass and anti-mosquito behavior, also affect host selection.[28–31]

In the present study, we tested whether *Cx. pipiens* mosquitoes feed selectively on certain avian hosts and avoid others, and whether these potential variations affected WNV transmission patterns in a known focus of arbovirus transmission.[32–34] By incorporating measures of host selection based upon assessment of host availability, we tested whether American robins are overused relative to other common species. Furthermore, we examined whether temporal patterns reflect a shift in feeding preferences from birds to mammals

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coincident with the onset of human WNV cases. Finally, we modeled the amplification fraction (a measure of the number of infectious *Cx. pipiens* resulting from each bird species) to predict the relative contributions of different bird species to WNV maintenance and amplification.

MATERIALS AND METHODS

Study sites. Sampling sites were in suburban southwest Chicago, Illinois (Cook County; 87°44′W, 41°42′N) and included 11 residential sites and four semi-natural sites (three cemeteries and a wildlife refuge) in 2005 and an additional 10 residential sites and 1 natural site (a forest preserve) in 2006. In 2007, we returned to 10 of the same residential sites and 4 natural sites and added 5 residential sites. Selection criteria for study sites were previously described.[35] Human WNV case data, including date of onset and location, were provided by the Illinois Department of Public Health without personal identifiers. Human cases considered in this report occurred within a 5-km buffer around the 15 field sites in 2005, 26 field sites in 2006, and 19 field sites in 2007. Spatial data were processed using the ArcGIS 9.2 software (Environmental Systems Research Institute, Redland, CA).

Mosquito collections, species identification, and WNV infection rates. Mosquitoes were sampled from each study site once every two weeks from mid-May through mid-October in 2005–2007, using CO_2-baited Centers for Disease Control and Prevention (CDC) (Atlanta, GA) miniature light traps, CDC gravid traps baited with rabbit pellet infusion, and battery-powered backpack aspirators. Mosquitoes were identified to species morphologically[36] and blood-fed individuals were separated from gravid and unfed individuals. Non-bloodfed mosquitoes were pooled and tested for WNV RNA using reverse transcription, quantitative polymerase chain reaction (PCR).[35] For blood-fed mosquitoes, the abdomens were removed (see below), and the carcasses were tested for WNV RNA individually as above. Maximum likelihood estimates for infection rates were calculated using the Pooled Infection Rate version 3.0 add-in[37] in the program Excel (Microsoft, Redmond, WA). Blood-fed *Culex* spp. mosquitoes were identified to species using a PCR-based method.[38]

Blood meal analysis. The relative amount of blood in the abdomens from blood-fed mosquitoes was scored with the Sella scale (1 = unfed; 2–6 = partial to full blood meal; 7 = gravid[39]). Using sterile technique, we removed the abdomen from each specimen, transferred it to a microcentrifuge tube, and DNA was extracted from it (DNeasy Tissue Kits; Qiagen, Valencia, CA). Extracted DNA served as template for a series of PCRs using primer pairs complementary to nucleotide sequences of the vertebrate cytochrome b (*cyt b*) gene as follows. Each sample was tested in two reactions using two separate primer pairs, one termed avian a (5′-GAC TGT GAC AAA ATC CCN TTC CA-3′ and 5′-GGT CTT CAT CTY HGG YTT ACA AGA C-3′;) and the other termed mammal a (5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′ and 5′-TGT AGT TRT CWG GGT CHC CTA-3′).[40] The Failsafe PCR System (Epicentre Biotechnologies, Madison, WI) was used, and conditions consisted of an initial denaturation for 3.5 minutes at 95°C, followed by 36 cycles consisting of denaturation (30 seconds at 95°C), annealing (50 seconds at 60°C), extension (40 seconds at 72°C), and a final extension for 5 minutes at 72°C. Amplicons were visualized by electrophoresis (E-gel system; Invitrogen, Carlsbad, CA), scored by band intensity (0 = no product; 5 = bold product), and purified (QIAquick PCR Purification Kits; Qiagen).

Nucleotide sequences of amplicons were obtained by direct sequencing (ABI Prism 3700 DNA Analyzer; Applied Biosystems, Foster City, CA). Sequences were subjected to BLAST search in GenBank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Returns to searches were evaluated as follows. Each chromatogram was inspected (Chromas Lite software; Technelysium Pty. Ltd., Tewantin, Queensland, Australia) for sequence quality and presence of double-nucleotide peaks, which may indicate blood from more than one vertebrate species in the blood meal.[41] Samples that produced an amplicon in one or the other reaction and a satisfactory match by BLAST were accepted as the likely host of origin, typically with 99% sequence match. Samples that did not produce an amplicon after the first two reactions, and amplicons that yielded ambiguous sequences (low-quality or double-nucleotide peaks), were subjected to a third PCR using the BM primer pair (5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′ and 5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′) under reaction conditions described above.[40,41] Samples that did not produce an amplicon or yielded ambiguous sequences in the third reaction (BM primer set) were subjected to a final round of PCR using a primer pair designed for reptiles and amphibians (i.e., herp) (5′-GCH GAY ACH WVH HYH GCH TTY TCH TC-3′ and 5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′).[42] Reaction conditions for the herp primer pair consisted of an initial denaturation for 2 minutes at 95°C, followed by 55 cycles consisting of denaturation (45 seconds at 94°C), annealing (50 seconds at 50°C), extension (1 minute at 72°C), and a final extension for 7 minutes at 72°C. Nucleotide sequences from amplicons of the BM and herp PCRs were similarly obtained and submitted for BLAST, and the likely host was determined by best match to the GenBank database. A blood meal was classified as mixed if two different species were identified in two separate PCRs from the same template and when chromatograms from each PCR demonstrated double-nucleotide peaks.

Sterile technique was used during preparation and handling of abdomens and for DNA extraction. Instruments were autoclaved and subjected to at least one hour of germicidal light prior to use. Negative controls were used during all steps (DNA extraction, PCRs, PCR product clean-up, and sequencing) to monitor for contamination. Positive controls of known-origin blood (16 species of birds, 8 species of mammals, and 2 species of amphibians) were processed and correctly identified with the above procedures. Species selected as controls were known to occur in the study region, and included American robin, American goldfinch (*Carduelis tristis*), brown-headed cowbird (*Molothrus ater*), blue jay (*Cyanocitta cristata*), European starling (*Sturnus vulgaris*), pied-billed grebe (*Podilymbus podiceps*), house sparrow (*Passer domesticus*), red-winged blackbird (*Agelaius phoeniceus*), wood thrush (*Hylocichla mustelina*), northern cardinal (*Cardinalis cardinalis*), song sparrow (*Melospiza melodia*), warbling vireo (*Vireo gilvus*), house finch (*Carpodacus mexicanus*), gray catbird (*Dumetella carolinensis*), orchard oriole (*Icterus spurius*), common grackle (*Quiscalus quiscula*), human (*Homo sapiens*), raccoon (*Procyon lotor*), domestic cat (*Felis catus*), white-footed mouse (*Peromyscus leucopus*), striped skunk (*Mephitis mephitis*), fox squirrel (*Sciurus niger*), eastern cottontail (*Sylvilagus floridanus*), Virginia opossum (*Didelphis*

virginiana), American toad (*Bufo americanus*), and American bullfrog (*Rana catesbeiana*). DNA was extracted from 5 μL of either whole blood or from blood clots to simulate a similar quantity of blood in a mosquito abdomen.

Bird survey. Local bird abundance was quantified at each site twice in 2005 and 2006 using survey point counts as previously described.[43] Briefly, five points were established in each residential site and eight in each natural site. We conducted all surveys between 0.5 hours before sunrise and 4.0 hours after sunrise (5:30 AM–10:00 AM) on days with no precipitation and wind speed less than 24 km/hour. Surveys were conducted between June and mid-July, corresponding with the peak avian breeding season in the region. In 2005, five of 11 residential and all four natural sites were surveyed. In 2006, all 21 residential and five natural sites were surveyed. Five-minute unlimited radius point counts were conducted at each survey point, distance to each observed bird was recorded, and density of each species and total avian density were estimated using Program Distance 5.0.[44]

In 2005, wild birds were captured using 36-mm mesh nylon mist-nets (Avinet, Inc., Dryden, NY) at each site six times at three-week intervals from mid-May to August and at five-week intervals in September and October. In 2006, the same rotation schedule was observed but eight additional residential sites were included. In 2007, 10 residential sites and three natural sites were sampled. Birds were identified to species, weighed, measured, aged, and sexed, and banded with numbered U.S. Fish and Wildlife Service leg bands (U.S. Department of Interior Bird Banding Laboratory, Federal Bird Banding Permit #06507). All fieldwork was carried out under appropriate collecting permits with approvals from the Institutional Animal Care and Use Committee at Michigan State University, Animal Use Form No. 2/03-152-00 and University of Illinois at Urbana-Champaign Animal Use Protocol No. 03034.

Calculation of host preference. Host feeding preferences for birds were calculated using the Manly resource selection design II index,[45] a ratio in which the use of resources is measured for individual mosquitoes and host availability is measured at the population level. Statistics were estimated using the adehabitat package in Program R.[46] The Manly selection ratio uses relative density as the measure of host availability (density-based selection ratio; $\hat{w}_i$) and was calculated for *Cx. pipiens*, *Cx. restuans*, and comparatively for *Cx. pipiens* from residential and natural sites as follows

$$\hat{w}_i = \frac{\text{proportion of utilized bird species } i}{\text{proportion of available bird species } i} = \frac{o_i}{\hat{\pi}_i}$$

A selection ratio of one represents the condition when mosquito feeding on host *i* is in equal proportion to estimated availability. A selection ratio greater than one represents overuse (i.e., more frequent feeding than expected by chance), and a ratio less than one represents underuse (i.e., less frequent feeding than expected by chance). The standard error of $\hat{w}_i$ was estimated as follows

$$\text{SE } (\hat{w}_i) = \sqrt{\left(\frac{o_i}{\hat{\pi}_i}\right)^2 * \left[\left(\frac{\text{var } o_i}{o_i^2}\right) + \left(\frac{\text{var}\hat{\pi}_i}{\hat{\pi}_i^2}\right)\right]}$$

The available resource units (i.e., birds by species) were estimated and the total number of census points (n = 145) was used to calculate the variance of $\hat{\pi}_i$ for a conservative measure of host availability (var $\hat{\pi}_i = \hat{\pi}_i * (1 - \hat{\pi}_i)$/sum (available hosts = 145)). Overuse or underuse for a host species was considered statistically significant when the 95% confidence interval (CI) did not overlap unity.

The selection index (w_i) was calculated for *Cx. pipiens* separated by trap type (light, gravid, aspirator), as well as for all individuals combined. Spatial comparison of host selection indices was conducted by calculating the selection index (w_i) for *Cx. pipiens* in residential sites and in natural sites. This analysis separated blood meal results and relative avian densities for residential and natural sites. When calculating feeding preferences, bird species that were not observed as blood meal hosts but were identified in bird surveys were given a blood meal value of one. Bird species observed as blood meal hosts but not identified in bird surveys were given a density equal to the lowest observed bird density, which was 0.0007 birds/hectare.

Amplification fraction. The amplification fraction for each bird species included in the analysis was modeled to integrate host selection ratios and host competence values and to provide a measure of importance for different bird species in the transmission of WNV[6] using a function modified by A. M. Kilpatrick (unpublished data). Competence values were obtained from Kilpatrick and others.[1] The amplification fraction (F_i) represents the estimated proportion of WNV infectious mosquitoes whose infection resulted from feeding on an individual of a certain bird species. It is estimated as the product of the relative avian abundance of host i (a_i), feeding preference of host i (P_i), and competence of host i (C_i), where P_i is a different measure of host selection compared with the Manly selection ratio described above. P_i incorporated the fraction of total avian and mammalian blood meals instead of just avian blood meals.

$$P_i = \frac{\text{fraction of total blood meals from host } i}{(\text{density of species } i \text{ /total avian density}) \ i} = \frac{B_i}{a_i}$$

The probability of each species becoming infected is proportional to the feeding preference, P_i, which changes the amplification fraction to $F_i = a_i \times P_i \times P_i \times C_i$. This expression reduces to $F_i = B_i \times P_i \times C_i$. The amplification fraction was calculated for host availability measures using relative avian densities (F_i). The amplification fraction assumes equal initial seroprevalence, and equal feeding preferences and competence values on adult and juvenile birds. Bird species without a host-competence index were assigned the average competence value for their respective family because more variation occurs between taxonomic families of birds than within them.[6] Because several species did not have a member of its respective family with a known competence value, the average competence for the respective avian order was assigned (Passeriform = 0.773).

RESULTS

Mosquito collections, species identification, and WNV infection rates. A total of 1,483 bloodfed mosquitoes were collected in 2005–2007, representing nine species (Table 1). Identification of *Culex* spp. by PCR resulted in an interpretable result in 91.8% of specimens, where *Cx. pipiens* was the most common *Culex* spp. mosquito (69.2%), *Cx. restuans* next common (22.4%), and the remainder (8.2%) were identified only as *Culex* spp. except

TABLE 1
Number and percentage of blood meals by host class for mosquitoes collected from suburban southwest Chicago, Illinois, 2005–2007

Taxon	Avian (%)	Mammal (%)	Amphibian (%)	Mixed			Total
				Avian–avian (%)	Mammal–mammal (%)	Avian–mammal (%)	
Culex pipiens	488 (80)	98 (16)		6 (1)	4 (1)	15 (2)	611
Cx. restuans	172 (81)	31 (15)	1 (< 1)	3 (1)		6 (3)	213
Cx. salinarius		1 (100)					1
Culex spp.	37 (71)	13 (25)	2 (4)				52
Anopheles quadrimaculatus		2 (100)					2
Culiseta inornata	1 (50)	1 (50)					2
Aedes vexans	15 (11)	111 (80)		1 (1)	9 (6)	3 (2)	139
Coquillettidia perturbans	1 (25)	2 (50)				1 (25)	4
Ochlerotatus triseriatus		5 (100)					5
Oc. trivittatus	1 (7)	13 (93)					14

for two individual *Cx. salinarius*. For all mosquito species of all genera, *Cx. pipiens* predominated in collections (57%), *Cx. restuans* was next in abundance (19%), and *Aedes vexans* (14%) was third in rank abundance. West Nile virus RNA was detected in 14 individual mosquitoes, including 12 *Cx. pipiens* and 2 unidentified *Culex* spp., yielding an infection rate of 18/1,000 in 2005, 7.4/1,000 in 2006, and 8.09/1,000 in 2007.

Blood meal analysis. The hosts of the blood meals of 1,043 (70%) of 1,483 mosquitoes were identified (Table 1). The proportion of reactions yielding amplicons and sequences decreased with increasing Sella score (R^2 = 0.91, degrees of freedom [df] = 4, P = 0.002). Blood meals from *Cx. pipiens* (comprising the bulk of the sample) were identified most commonly as avian (n = 488, 80%), and less commonly but not infrequently as mammalian (n = 98, 16%). A small number were of mixed source (n = 25, 4%, Table 1). Blood meals from *Cx. restuans* were also most commonly (85%) identified to an avian host. Blood meals from *Aedes*, *Anopheles*, and *Ochlerotatus* mosquitoes were primarily identified as mammal hosts (80%, 100%, and 93–100% of blood meals, respectively), but 11% of blood meals from *Ae. vexans* were of avian origin.

Results of BLAST searches of *cyt b* sequences showed that *Cx. pipiens* fed upon 25 avian species with the most common being American robin (48% of avian blood meals), house sparrow (15%), mourning dove (*Zenaida macroura*; 11%), and northern cardinal (8%, Table 2). Results from *Cx. restuans* were similar in the pattern of host feeding, but only 18 bird species were identified. Results showed that among the mammals fed upon by *Cx. pipiens*, the most common were humans (83% of mammalian blood meals), and raccoons (8%, Table 3). Of those blood meals identified as mammalian in *Cx. restuans*, most were from human (84%) but also included raccoon (8%), and eastern cottontail (5%). Mammalian blood meals from *Ae. vexans* were mostly white-tailed deer (*Odocoileus virginianus*; 48%), human (31%), and eastern cottontail (14%). No reptile blood meals were observed and the only amphibian hosts included one *Cx. restuans* and two *Culex* spp. mosquito that were found to have fed upon gray treefrogs (*Hyla versicolor*). Two percent of *Cx. pipiens* with mixed blood meals contained blood from birds and mammals.

Bird abundance. A total of 44 avian species were identified during point count surveys with a total density of 9.66 birds/hectare. House sparrows (4.25 birds/hectare), American robins (2.0 birds/hectare), mourning doves (0.63 birds/hectare), common grackles (0.56 birds/hectare), and European starlings (0.55 birds/hectare) were the most common species. A total of 1,407 birds of 57 species were captured in mist nets in 2005, 1,479 birds of 63 species in 2006, and 1,377 birds of 51 species in 2007. The most commonly captured species were the house sparrow (combined years n = 1,461), American robin (n = 693), American goldfinch (n = 292), gray catbird (n = 277), and northern cardinal (n = 230).

Host preference. The host selection ratio varied among the different avian species found to have been fed upon by *Cx. pipiens* (Table 4). Of the species for which the selection ratio was greater than 1 (indicating overuse relative to availability), the American robin ($\hat{w}_i$ = 2.81) was the only host for which the ratio was statistically significant (95% CI = 1.17–4.46) when calculated for individuals collected with aspirators. American robins were marginally significantly overused when all *Cx. pipiens* were combined (2.26; 95% CI = 0.98–3.54). Of the species for which the selection ratio was less than one (indicating underuse), the statistically significant species were common grackle ($\hat{w}_i$ = 0.06), red-winged blackbird (0.08), American goldfinch (0.09), monk parakeet (*Myiopsitta monachus*; 0.11), house sparrow (0.32), and European starling (0.39). *Culex restuans* feeding preferences displayed similar overall host selection, but no bird species were significantly overused and only three were significantly underused (American goldfinch, 0.22; common grackle, 0.24; and house sparrow, 0.33; Table 5).

Selection ratios for *Cx. pipiens* between residential and natural sites were significantly different (t = 3.67, df = 48, P < 0.001). Overuse was higher for several species in residential sites than natural sites, including mallard (39.9 ± 451, 0.2 ± 0.2; respectively; Table 6) and American robin (2.4 ± 0.4, 1.2 ± 0.2). Underuse was stronger for house sparrow (0.3 ± 0.04, 0.4 ± 0.2) and common grackle (0.1 ± 0.05, 0.3 ± 0.36) in residential sites than in natural sites.

The abundance of American robins captured using mist nets decreased as the summer season progressed, and the abundance of house sparrows in mist nets increased by comparison (Figure 1A). The proportion of *Cx. pipiens* feeding on American robins decreased as the season progressed (Figure 1B), and concomitantly there was an increase in feeding on other avian species, such as house sparrow, mourning dove, and northern cardinal (Figure 1B).

Epidemic curve. A total of 2,753 pools (53,230 individuals) of non-bloodfed *Culex* mosquitoes from 2005–2007 were tested for WNV RNA; 519 (18.9%) of the pools were positive and the peak infection rate (21.9/1,000 individuals) occurred in August. *Culex pipiens* infection with WNV and abundance peaked during the months of August and September, respectively

TABLE 2

Number and percentage of blood meals identified to avian or mixed avian hosts for mosquitoes collected in suburban southwest Chicago, Illinois, 2005–2007*

Host	Fraction of species I in avian community	Mosquito species			
		Culex pipiens (%)†	*Cx. restuans* (%)‡	*Culex* spp. (%)§	*Aedes vexans* (%)¶
American goldfinch	0.0214	1 (< 1)			
American kestrel	0.0001#	3 (1)			
American robin	0.2026	249 (48)	83 (45)	20 (54)	12 (60)
Black-capped chickadee	0.0020	2 (< 1)			
Blue jay	0.0030	14 (3)	2 (1)		
Brown-headed cowbird	0.0028		2 (1)		
Brown thrasher	0.0001#	1 (< 1)			
Cedar waxwing	0.0062	2 (< 1)			
Chicken	0.0001#				1 (5)
Chipping sparrow	0.0080	2 (< 1)			1 (5)
Common canary	0.0001#	1 (< 1)			1 (5)
Common grackle	0.0576	2 (< 1)	3 (2)	2 (5)	
Cooper's hawk	0.0001#	1 (< 1)			
Eastern bluebird	0.0002		1 (1)		
Eastern towhee	0.0002			1 (3)	
European starling	0.0567	12 (2)	11 (6)		
Field sparrow	0.0001#		1 (1)		
Gray catbird	0.0047	2 (< 1)	2 (1)	1 (3)	
House finch	0.0110	34 (7)	8 (4)	1 (3)	1 (5)
House sparrow	0.4400	76 (15)	31 (17)	3 (8)	1 (5)
House wren	0.0030	3 (1)			
Mallard	0.0091		1 (1)		
Mourning dove	0.0650	55 (11)	10 (5)	4 (11)	1 (5)
Northern cardinal	0.0144	43 (8)	19 (10)	4 (11)	
Northern flicker	0.0003	1 (< 1)			
Red-winged blackbird	0.0454	2 (< 1)	4 (2)		
Rock pigeon	0.0095	1 (< 1)			
Scarlet tanager	0.0002	3 (1)	3 (2)		1 (5)
Song sparrow	0.0017	2 (< 1)	1 (1)	1 (3)	
Swainson's thrush	0.0001#	2 (< 1)			
Swamp sparrow	0.0001#		1 (1)		
Turkey	0.0001#				1 (5)
Veery	0.0006	1 (< 1)	1 (1)		
Total avian-derived blood meals		515	184	37	20

* Avian relative abundance provided as the fraction of species i in the avian community (density of species i/total avian density).
† Includes 27 specimens from which double blood meals were identified.
‡ Includes 12 specimens from which double blood meals were identified.
§ *Culex* mosquitos that did not produce a polymerase chain reaction amplicon using the *Culex* spp. primer sets (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*).
¶ Includes 5 specimens from which double blood meals were identified.
\# Species was not observed during surveys and was given lowest observed bird density for analysis.

(Figure 2A). Seventy-six human cases of WNV infection were reported within 5 km of the field sites in 2005–2007, and peak date of onset occurred in August (Figure 2B). When human exposure to WNV peaked, there was a high percentage of bird feeding by *Cx. pipiens* and a smaller fraction of feeding on mammals, including humans (Figure 2B).

TABLE 3

Number and percentage of blood meals identified to mammal or mixed mammal hosts for mosquitoes collected in suburban southwest Chicago, Illinois, 2005–2007

Host	Mosquito species			
	Culex pipiens (%)*	*Cx. restuans* (%)†	*Culex* spp. (%)‡	*Aedes vexans* (%)§
Cat	2 (2)			1 (1)
Domestic dog	1 (1)			2 (2)
Human	100 (83)	31 (84)	8 (62)	41 (31)
Opossum	3 (2)			2 (2)
Eastern cottontail		2 (5)		19 (14)
Raccoon	10 (8)	3 (8)	1 (8)	3 (2)
Gray squirrel	3 (2)	1 (3)	1 (8)	
White-tailed deer	2 (2)		3 (23)	64 (48)
Total mammal-derived blood meals	121	37	13	132

* Includes 23 specimens from which double blood meals were identified.
† Includes 6 specimens from which double blood meals were identified.
‡ *Culex* mosquitos that did not produce a polymerase chain reaction amplicon using the *Culex* spp. primer sets (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*).
§ Includes 21 specimens from which double blood meals were identified.

There was statistically significant temporal variation in the frequency of bird and mammal feeding by *Cx. pipiens* (2 × 5 contingency table, $\chi^2 = 24.05$, df = 4, $P < 0.0001$) (Figure 2B). Mammal feeding was proportionately higher in June and September, deviating strongly from expectation by chance alone (+24.6% and +51.6% deviation, respectively), and was proportionately lower in July, August, and September, also deviating negatively from chance alone (–16%, –18.1%, and –11.8% deviation, respectively). The variation in bird and human feeding by month was also significant ($\chi^2 = 20.2$, df = 4, $P = 0.0005$) with similar higher feeding on humans in May and September (+37% and +88.5% deviation, respectively).

Amplification fraction. Species-specific amplification fractions were estimated by incorporating the abundance of birds of different species, and their known reservoir competence, into the selection. Results indicate that American robins accounted for 35% of the WNV infections in *Cx. pipiens*, blue jays accounted for 17%, and house finches accounted for 15%, American kestrel (*Falco sparverius*) accounted for 11%, and northern cardinal accounted for 5% (Figure 3). Together,

TABLE 4
Host-feeding preferences of *Culex pipiens* collected in suburban southwest Chicago, 2005–2007, in total and broken down by trap type

Host	*Cx. pipiens* feeding preference (standard error)			
	Total $\hat{w}_i$	Light trap $\hat{w}_i$	Gravid trap $\hat{w}_i$	Aspirator $\hat{w}_i$
American kestrel*	75.51 (735.08)	161.10 (1,573.70)	75.65 (737.09)	73.62 (719.16)
Swainson's thrush*	50.34 (490.49)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Scarlet tanager	34.09 (223.39)	72.72 (480.25)	51.22 (335.70)	33.23 (219.47)
Brown thrasher*	24.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Common canary*	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Cooper's hawk*	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Ring-necked pheasant†	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Hairy woodpecker†	12.96 (91.29)	82.95 (584.27)	19.48 (137.19)	37.91 (267.00)
Eastern towhee†	11.85 (79.86)	75.82 (511.09)	17.80 (120.01)	34.65 (233.56)
Eastern bluebird†	10.75 (69.06)	68.77 (441.99)	16.15 (103.78)	31.43 (201.99)
Blue jay	8.44 (12.88)	3.86 (6.97)	10.88 (16.63)	1.76 (3.18)
Willow flycatcher†	7.17 (37.87)	45.89 (242.37)	10.78 (56.91)	20.97 (110.76)
Common yellowthroat†	6.95 (36.12)	44.45 (231.19)	10.44 (54.29)	20.31 (105.65)
House finch	5.69 (4.58)	5.35 (4.82)	6.03 (4.90)	2.45 (2.21)
Northern cardinal	5.50 (3.87)	3.27 (2.77)	6.72 (4.75)	1.50 (1.27)
Northern flicker†	5.40 (24.87)	34.54 (159.19)	8.11 (37.38)	15.78 (72.75)
Killdeer†	4.68 (20.14)	29.93 (128.90)	7.03 (30.27)	13.68 (58.90)
Eurasian collared-dove†	4.65 (19.95)	29.74 (127.68)	6.98 (29.98)	13.59 (58.35)
Eastern kingbird†	4.04 (16.25)	25.87 (104.01)	6.08 (24.42)	11.82 (47.53)
Great-crested flycatcher†	3.64 (13.91)	23.27 (89.00)	5.46 (20.90)	10.63 (40.67)
Warbling vireo†	3.54 (13.35)	22.63 (85.43)	5.31 (20.06)	10.34 (39.04)
White-breasted nuthatch†	3.29 (12.00)	21.04 (76.78)	4.94 (18.03)	9.61 (35.09)
Veery	3.12 (11.13)	19.98 (71.24)	4.69 (16.73)	9.13 (32.56)
Indigo bunting†	3.09 (10.96)	19.77 (70.15)	4.64 (16.47)	9.04 (32.06)
Eastern wood-pewee†	2.67 (8.84)	17.06 (56.57)	4.01 (13.28)	7.80 (25.85)
Red-eyed vireo†	2.49 (7.99)	15.91 (51.11)	3.74 (12.00)	7.27 (23.36)
Yellow warbler†	2.27 (7.02)	14.55 (44.90)	3.42 (10.54)	6.65 (20.52)
American robin	2.26 (0.39)	0.64 (0.21)	1.80 (0.32)	2.81 (0.50)‡
Song sparrow	2.11 (4.45)	6.75 (15.02)	3.17 (6.69)	3.09 (6.87)
Barn swallow†	1.94 (5.59)	12.44 (35.81)	2.92 (8.41)	5.68 (16.36)
Black-capped chickadee	1.86 (3.71)	5.95 (12.61)	1.49 (2.96)	2.72 (5.76)
House wren	1.82 (2.95)	3.89 (7.04)	0.91 (1.65)	1.78 (3.22)
Blue-gray gnatcher†	1.81 (5.05)	11.58 (32.31)	2.72 (7.59)	5.29 (14.77)
Mourning dove	1.55 (0.53)	1.27 (0.61)	1.74 (0.61)	0.58 (0.28)
Baltimore oriole†	1.12 (2.55)	7.15 (16.29)	1.68 (3.83)	3.27 (7.45)
Gray catbird	0.78 (1.09)	2.49 (3.90)	1.17 (1.63)	1.14 (1.78)
Brown-headed cowbird†	0.65 (1.21)	4.19 (7.77)	0.98 (1.82)	1.91 (3.55)
Cedar waxwing	0.59 (0.75)	1.89 (2.74)	0.89 (1.12)	0.86 (1.25)
American crow†	0.54 (0.93)	3.45 (5.98)	0.81 (1.41)	1.58 (2.73)
Downy woodpecker†	0.53 (0.91)	3.39 (5.85)	0.80 (1.37)	1.55 (2.68)
Chipping sparrow	0.46 (0.54)	1.48 (2.01)	0.69 (0.81)	0.67 (0.92)
European starling	0.39 (0.17)‡	0.21 (0.22)‡	0.39 (0.19)	0.28 (0.19)‡
House sparrow	0.32 (0.05)‡	0.24 (0.08)‡	0.34 (0.05)‡	0.16 (0.05)‡
Mallard†	0.20 (0.27)	1.29 (1.71)	0.30 (0.40)	0.59 (0.78)
Rock pigeon	0.19 (0.25)	1.24 (1.62)	0.29 (0.38)	0.57 (0.74)
Monk parakeet†	0.11 (0.13)‡	0.70 (0.82)	0.16 (0.19)‡	0.32 (0.38)
American goldfinch	0.09 (0.10)‡	0.55 (0.63)	0.13 (0.15)‡	0.25 (0.29)
Red-winged blackbird	0.08 (0.07)‡	0.26 (0.28)	0.12 (0.10)‡	0.12 (0.13)‡
Common grackle	0.06 (0.05)‡	0.20 (0.22)‡	0.10 (0.07)‡	0.09 (0.10)‡

* Species not recorded during avian surveys (given value of the lowest observed bird density).
† Species not observed as a host in the blood meal analysis (given a value of 1).
‡ Statistically significant non-rando m host selection at $P < 0.05$.

these five species accounted for 82% of the WNV-infectious *Cx. pipiens*.

DISCUSSION

The amplification of WNV infection in mosquitoes and bridging of transmission to humans resulting in human infection and disease are intertwined processes whose intensity depends upon the interaction of mosquito vector and vertebrate host populations. On the basis of longitudinal population analyses in three consecutive seasons in the Chicago study region, we have concluded that *Cx. pipiens* functions as both the epizootic and epidemic (i.e., bridge) vector,[47,48] and that the annual flush of nestling and fledgling birds is a causative factor in seasonal amplification.[35] In the present study, two primary questions were considered: first, what birds (or other animals) are serving as the blood hosts of this mosquito vector, and second, does variation in blood host use influence amplification and bridging transmission?

Our results document extensive feeding of *Cx. pipiens* on humans. This finding is especially striking because this species is thought to rely primarily upon avian hosts for blood. Yet, the results of this study do not support the hypothesis that a shift in *Cx. pipiens* feeding from birds to mammals correlates with elevated human risk of infection, a phenomenon observed elsewhere[7] and attributed to a seasonal decline in

TABLE 5
Host-feeding preferences of *Culex restuans* collected in suburban southwest Chicago, Illinois, 2005–2007

Host	*Cx. restuans* feeding preference $\hat{w}_i$ (standard error)
Scarlet tanager	87.00 (570.02)
Field sparrow*	64.25 (627.45)
Ring-necked pheasant†	64.25 (627.45)
Swamp sparrow*	64.25 (627.45)
Hairy woodpecker†	33.08 (232.96)
Eastern towhee†	30.24 (203.78)
Eastern bluebird	27.43 (176.23)
Willow flycatcher†	18.30 (96.64)
Common yellowthroat†	17.73 (92.18)
Northern flicker†	13.78 (63.47)
Killdeer†	11.94 (51.39)
Eurasian collared-dove†	11.86 (50.91)
Eastern kingbird†	10.32 (41.47)
Great-crested flycatcher†	9.28 (35.49)
Warbling vireo†	9.02 (34.06)
White-breasted nuthatch†	8.39 (30.61)
Veery	7.97 (28.41)
Indigo bunting†	7.89 (27.97)
Eastern wood-pewee†	6.80 (22.55)
Red-eyed vireo†	6.35 (20.38)
Northern cardinal	6.20 (4.48)
Yellow warbler†	5.80 (17.90)
Barn swallow†	4.96 (14.28)
Blue-gray gnatcher†	4.62 (12.88)
House finch	3.42 (2.94)
Brown-headed cowbird	3.34 (5.73)
Blue jay	3.08 (5.11)
Baltimore oriole†	2.85 (6.50)
Song sparrow	2.69 (5.99)
Black-capped chickadee†	2.37 (5.03)
Gray catbird	1.99 (2.78)
American robin	1.92 (0.36)
House wren†	1.55 (2.81)
American crow†	1.37 (2.39)
Downy woodpecker†	1.35 (2.33)
European starling	0.91 (0.41)
Mourning dove	0.80 (0.34)
Cedar waxwing†	0.75 (1.09)
Chipping sparrow†	0.59 (0.80)
Mallard	0.51 (0.68)
Rock pigeon†	0.49 (0.65)
Red-winged blackbird	0.41 (0.26)
House sparrow	0.33 (0.06)‡
Monk parakeet†	0.28 (0.33)
Common grackle	0.24 (0.16)‡
American goldfinch†	0.22 (0.25)‡

* Species not recorded during avian surveys (given value of the lowest observed bird density).
† Species not observed as a host (given a value of 1).
‡ Statistically significant non-random host selection at $P < 0.05$.

TABLE 6
Host-selection ratios for *Culex pipiens* collected in residential and natural study sites in suburban southwest Chicago, Illinois, 2005–2007

Host	*Cx. pipiens* feeding preference (standard error)	
	Residential sites $\hat{w}_i$	Natural sites $\hat{w}_i$
American kestrel*†‡	524.97 (12,372.68)	48.81 (326.50)
Scarlet tanager*†	524.97 (12,372.68)	8.75 (26.01)
Swainson's thrush*†‡	349.98 (8,249.70)	48.81 (326.50)
Brown thrasher†‡	174.99 (4,126.71)	48.81 (326.50)
Cooper's hawk*†‡	174.99 (4,126.71)	48.81 (326.50)
Eastern bluebird*†§	174.99 (4,126.71)	8.27 (24.00)
Eastern towhee*†	174.99 (4,126.71)	9.12 (27.63)
Yellow-shafted flicker*	174.99 (4,126.71)	48.81 (326.50)
Ring-necked pheasant*§	174.99 (4,126.71)	22.50 (103.54)
Swamp sparrow§	174.99 (4,126.71)	48.81 (326.50)
Veery*†§	174.99 (4,126.71)	2.40 (4.27)
Warbling vireo*†§	174.99 (4,126.71)	2.72 (5.04)
Willow flycatcher*†§	174.99 (4,126.71)	5.52 (13.46)
Yellow warbler*§	124.45 (2,475.79)	1.78 (2.87)
Mallard*§	39.92 (450.96)	0.16 (0.17)¶
Barn swallow*§	31.45 (315.66)	1.59 (2.48)
Common yellowthroat*§	27.64 (260.26)	7.06 (19.10)
White-breasted nuthatch*§	26.95 (250.64)	2.87 (5.42)
Hairy woodpecker*§	20.32 (164.36)	25.95 (127.78)
Killdeer*§	20.04 (160.99)	4.65 (10.56)
Eastern kingbird*§	17.15 (127.61)	4.03 (8.65)
Indigo bunting*§	16.96 (125.53)	2.89 (5.46)
Great-crested flycatcher*§	14.47 (99.05)	3.70 (7.67)
Blue jay	10.67 (18.16)	2.75 (3.60)
Baltimore oriole*§	10.11 (58l.15)	0.96 (1.31)
Blue-gray gnatcher*§	7.20 (35.16)	1.84 (2.99)
Northern cardinal	5.19 (3.69)	4.62 (3.45)
House finch	4.95 (3.73)	11.90 (18.13)
Eastern wood-pewee*§	4.87 (19.73)	4.35 (9.63)
Song sparrow*	4.76 (13.48)	1.42 (2.14)
Eurasian collared-dove*†§	4.48 (17.49)	48.81 (326.50)
Black-capped chickadee*	3.96 (10.32)	1.31 (1.93)
Red-eyed vireo*§	3.42 (11.77)	6.41 (16.64)
American robin	2.42 (0.44)	1.20 (0.21)
House wren	1.46 (2.44)	2.40 (4.25)
Mourning dove	1.31 (0.43)	2.57 (1.31)
Gray catbird§	1.04 (2.15)	0.94 (0.89)
Brown-headed cowbird*§	0.98 (1.98)	1.42 (2.14)
Cedar waxwing*	0.88 (1.22)	0.64 (0.80)
Red-winged blackbird*	0.79 (1.04)	0.03 (0.04)
Downy woodpecker*§	0.70 (1.26)	1.50 (2.30)¶
Chipping sparrow*	0.69 (0.86)	0.50 (0.61)
American crow*§	0.55 (0.90)	8.73 (25.92)
European starling	0.38 (0.18)¶	0.29 (0.19)¶
House sparrow	0.30 (0.04)¶	0.44 (0.20)
Rock pigeon*	0.19 (0.24)¶	48.81 (326.50)
American goldfinch§	0.13 (0.15)¶	0.19 (0.20)¶
Monk parakeet*§	0.12 (0.14)¶	0.69 (0.87)
Common grackle*	0.07 (0.05)¶	0.31 (0.36)

* Species not observed as a host in natural sites (given a value of 1).
† Species not recorded during avian surveys (given value of lowest observed bird density).
‡ Species not recorded during avian surveys (given value of lowest observed bird density).
§ Species not observed as a host in residential sites (given a value of 1).
¶ Statistically significant non-random host selection at $P < 0.05$.

bird availability (as opposed to some physiologic change affecting mosquito feeding patterns).[1] The initial high rate of feeding on American robin, also reported in other studies,[6,7] was followed by a gradual decrease in feeding on American robin (also reported in other studies[7,40]) supporting an interpretation of a broadly opportunistic strategy of *Cx. pipiens* where host availability of preferred hosts dictates the apparent feeding patterns reflected by blood meal analysis. This interpretation is supported by the similarity in feeding patterns exhibited by *Cx. restuans* (Tables 2 and 3). However, the decrease in feeding on robins was not accompanied by an increase in feeding on humans and other mammals, but rather by an increase in feeding on other bird species, in particular house sparrows, mourning dove, and northern cardinal (Figure 1B and 2B). Furthermore, the trend at the beginning and near the end of the season (June and September) was for a relatively higher frequency of feeding on mammals, but during the amplification events and dates of onset of human cases, frequency of feeding on mammals was actually significantly lower than the full season average and birds were the more frequent hosts. From these patterns, we conclude that the risk of human infection (i.e., bridging transmission) relates not to a shift in the bird: mammal ratio of feeding frequency, but rather to the amplification process itself. As the WNV infection rate in the *Cx. pipiens* population increases in July and August, some marginal virus transmission to humans occurs because of the fraction of the *Cx. pipiens* population

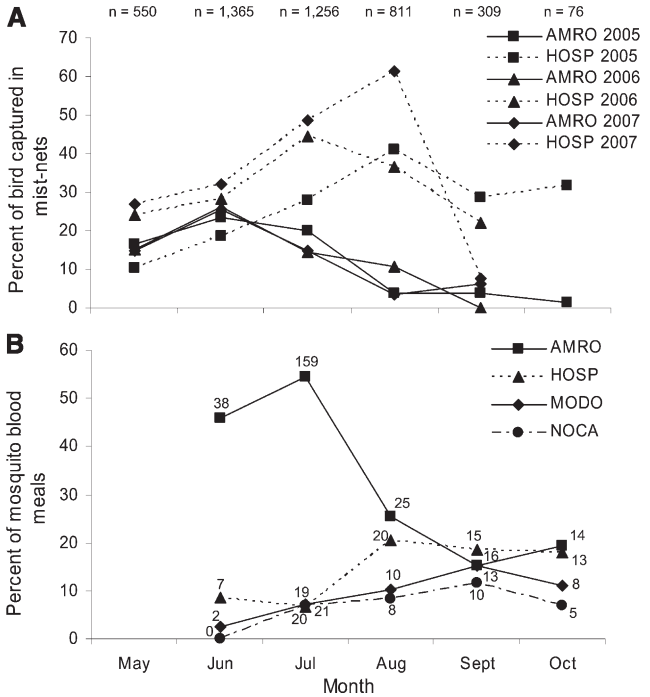

FIGURE 1. **A**, Percent of American robin and house sparrow captured in mist-nets in southwest suburban Chicago, Illinois, 2005–2007. **B**, Percent of *Culex pipiens* blood meals derived from American robin, house sparrow, mourning dove, and northern cardinal. Total sample size of birds captured in mist-nets for combined year are indicated in **A** and raw numbers are indicated for sample size in **B**.

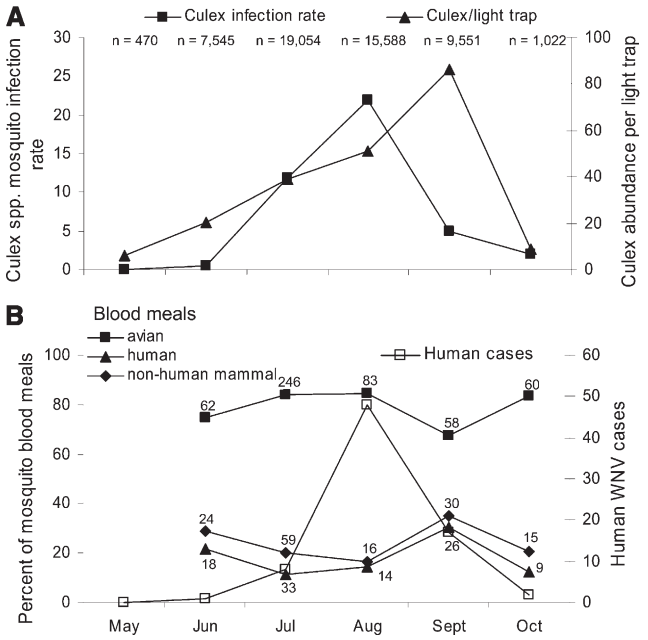

FIGURE 2. **A**, Temporal patterns of *Culex* spp. mosquito infection rate and abundance (*Culex* spp. per light trap) in southwest suburban Chicago, Illinois, 2005–2007. **B**, Percent of *Cx. pipiens* blood meals derived from birds, humans, and non-human mammals and human West Nile virus case date of onset in the same study sites during the same years. Raw numbers in **A** indicate total numbers of *Culex* spp. mosquitoes captured and tested. Mosquitoes captured in light traps are a subset of the total. Raw numbers in **B** indicate raw number of blood meals.

that during that time period bites humans. Given the sharp coincidence of amplification and dates of onset of human infection, interventions directed at processes promoting amplification seem paramount, especially those initiated immediately prior to and during generation of the epizootic curve.

Although host selection by *Cx. pipiens* and other *Culex* spp. was influenced by host availability, our analyses indicated that certain common species of birds were overused (American robin) or underused (common grackle, starling, house sparrow) relative to their abundance. The null hypothesis that *Cx. pipiens* selects avian blood hosts on the sole basis of relative availability was rejected. The behavioral and ecologic explanations for these patterns are unknown, but could relate to relative tendency of birds to aggregate into roosts, the position and structure of nests, the host-defensive behavior of nestlings and fledglings, and olfaction cues. Our results indicate that overuse of American robins, identified as a superspreader species because of its high reservoir competence, is not the sole determinant of intensification of WNV transmission during amplification. Simultaneous underuse of certain common species that have rather poor predicted reservoir competences (starlings and red-winged blackbirds in particular) similarly contributes to WNV amplification. This study indicates the house sparrow plays a minor role in amplification events although other studies have indicted this species as an important host for both St. Louis encephalitis virus[49] and WNV virus.[50] Here, there was less feeding on house sparrows than expected on the basis of their abundance, resulting in a lower amplification fraction. In contrast, the less common house finch was predicted to be an important amplifying host (Table 5 and Figure 3). It is also important to note that competence values used to calculate the amplification fraction are an aggregate of 11 primary research papers in which birds were experimentally infected.[1] Many avian species have yet to be the subject of such experimental studies, and many published competence values are based on small samples sizes of infected birds (e.g., American robin, n = 2). This limitation emphasizes the need for more experimental studies to complement field studies.

The presence of alternate avian hosts, after feeding on robins wanes, suggests that those birds might actually serve a zooprophylaxis function, as has been suggested for non-human mammal hosts (dogs, horses, and deer) in diverting infectious mosquitoes away from humans.[40,51] The same could be true for abundant avian hosts, especially ones with poor reservoir competence, which would serve to dampen transmission. This observation has important implications in the measure of host community competence and in understanding the so-called dilution effect.[43,52] Furthermore, it would offer an explanation for why WNV infection in *Cx. pipiens* decreases in August when temperatures are still supportive of transmission and birds remain generally available.

The differences in host selection in natural and residential sites within our relatively small study region demonstrate the importance of fine-scale variation in host availability. Stronger overuse for mallards and robins in residential sites than in natural sites indicates that *Cx. pipiens* host preference is context specific. The differences in these selection ratios are predicted to have dramatic effects on interpreting the contribution of birds to WNV transmission, and this finding might also provide a mechanism for high rates of transmission in suburban

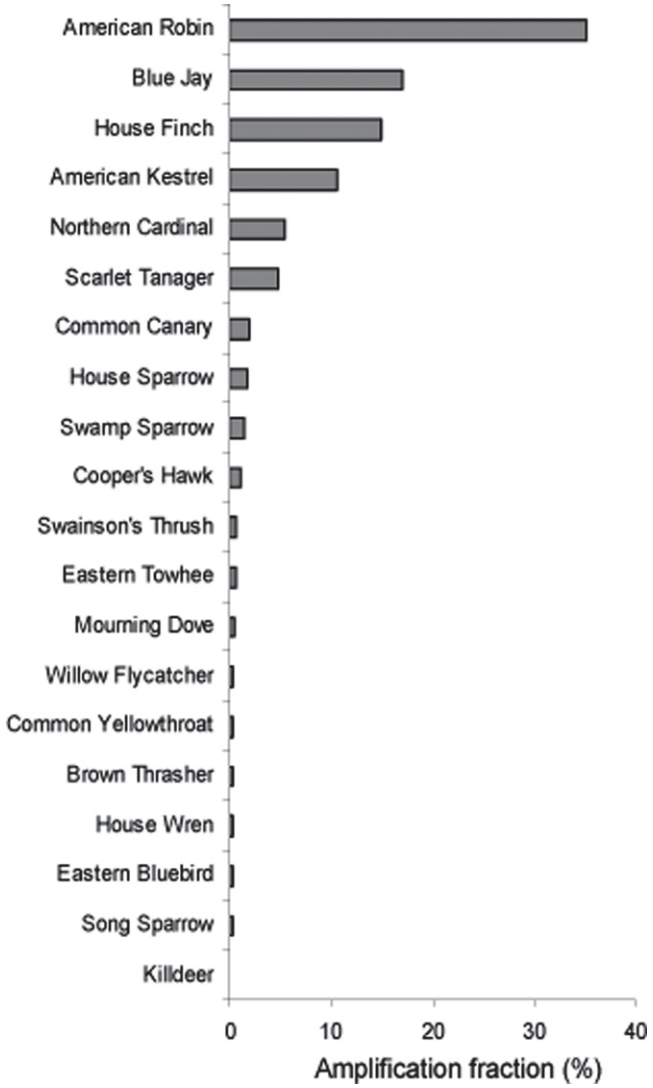

FIGURE 3. Amplification fraction (F_i) representing the fraction of West Nile virus infectious mosquitoes resulting from feeding on that avian host[1,6] (Kilpatrick AM, unpublished data). Species with amplification fractions < 0.02 are not graphed and include eastern kingbird, black-capped chickadee, great-creasted flycatcher, warbling vireo, white-breasted nuthatch, eastern wood-pewee, red-eyed vireo, hairy woodpecker, yellow warbler, barn swallow, blue-gray gnatcher, veery, indigo bunting, American crow, cedar waxwing, chipping sparrow, European starling, northern flicker, baltimore oriole, Eurasian collard-dove, common grackle, gray catbird, American goldfinch, mallard, downy woodpecker, red-winged blackbird, rock pigeon, monk parakeet, ring-necked pheasant, and brown-headed cowbird.

environments, where residential and natural areas are in close proximity.

The percent of avian feeding by *Cx. pipiens* varies considerably by region (35–96%).[7,16,40,53,54] We documented an unusually high rate of human feeding by *Cx. pipiens* (16% of total blood meals). Recent evidence confirms that a portion of this rate variation is genetically based. Specifically, population substructuring appears to exist in the *Cx. pipiens* complex, with an increased affinity for human hosts hypothesized for the *Cx. pipiens molestus* form.[55–58] A second hypothesis for variation in human feeding is host availability. Samples from residential areas such as alleys and residential backyards yielded 79% of the bloodfed *Cx. pipiens* in our study. Other recent blood meal analysis studies with *Cx. pipiens* were done within urban areas, but actual sample sites were parks, uninhabited military forts, sewage treatment plants, golf courses, cemeteries, woodlots, and public thoroughfares.[16,40,53,54] Collecting bloodfed mosquitoes in immediate proximity to human habitation could explain our finding of a high frequency of human feeding by *Culex* mosquitoes, a phenomenon supported by previous studies.[54,59,60]

We found that 4% of *Cx. pipiens* blood meals contained mixed sequences (more than one host species), which concords with a range of 3–8% reported in previous studies.[7,16,59,61] The direct sequencing method used in this study and others may overlook cryptic blood meals because of the amplification of the predominant blood meal, especially for species such as starlings with high anti-mosquito behavior,[62] which would be negatively biased. The overuse of robins by *Cx. pipiens* collected by aspirators and underuse of robins by *Cx. pipiens* collected in light traps suggests that host-seeking individuals with partial blood meals collected by light traps were less likely to contain robin blood than were those with a complete blood meal collected by aspirators. This finding is supported by the lower observed sella score, indicating a more complete, less digested blood meal, from aspirators, compared with those collected in light and gravid traps (3.2, 3.6, 4.1, respectively). Collectively, this supports the hypothesis that robins have relatively low anti-mosquito behavior, which enables *Cx. pipiens* to complete a blood meal.

Concurrent host-feeding and virus detection data for *Cx. pipiens* previously published[47] and the magnitude of bird feeding reinforces the role of *Cx. pipiens* as the primary enzootic vector in the study region. *Culex restuans* could also contribute to early-season enzootic transmission, but based on this sampling effort and molecular species identification, this species appears less important (*Cx. pipiens* are 3.1 times more abundant). The presence of a virus-positive *Cx. pipiens* with a human-derived blood meal demonstrates that this species is capable of being a bridge vector for epizootic transmission.[47] Host-feeding results for *Ae. vexans* showed more bird-feeding than we typically expect from this mammalophilic mosquito species.[16,53,63] Identification of 14% of *Ae. vexans* feeding on birds supports a recent study suggesting the potential role of this mosquito as a bridge vector.[48,64] During 2005–2007, this study collected 784 pools (11,701 individuals) of *Aedes vexans* but only 4 pools were positive for WNV RNA (infection rate of 0.34/1,000). Given the substantially lower infection rate compared with *Culex* spp. (infection rate of 11.03/1,000; 519 positive pools of 2,753), and the occurrence of a not insubstantial number of human cases at times and in places when *Ae. vexans* were absent, or present but uninfected, the role of *Ae. vexans* as a primary bridge vector seems unlikely. Indeed, relatively rare virus infection in *Ae. vexans* may reflect occasional feeding on infected robins but not significant vectorial capacity for WNV.

In this report, we present a modified expression for the amplification fraction (A. M. Kilpatrick, unpublished data), a measure of the avian species-specific contribution to WNV transmission. The finding that 66% (F_i) of WNV infectious *Cx. pipiens* became infected from feeding on viremic American robins (35%), blue jays (17%), and house finches (15%) combined implicates these common urban birds as the major contributors to epizootic transmission of WNV, in particular the force of infection.[65] The finding that these common urban birds may be responsible for WNV amplification provides a mechanism for this *Culex* spp. mosquito-driven disease system

to rapidly adapt to diverse bird communities during invasion and establishment across North America.

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Aquatic eutrophication promotes pathogenic infection in amphibians

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The widespread emergence of human and wildlife diseases has challenged ecologists to understand how large-scale agents of environmental change affect host–pathogen interactions. Accelerated eutrophication of aquatic ecosystems owing to nitrogen and phosphorus enrichment is a pervasive form of environmental change that has been implicated in the emergence of diseases through direct and indirect pathways. We provide experimental evidence linking eutrophication and disease in a multihost parasite system. The trematode parasite *Ribeiroia ondatrae* sequentially infects birds, snails, and amphibian larvae, frequently causing severe limb deformities and mortality. Eutrophication has been implicated in the emergence of this parasite, but definitive evidence, as well as a mechanistic understanding, have been lacking until now. We show that the effects of eutrophication cascade through the parasite life cycle to promote algal production, the density of snail hosts, and, ultimately, the intensity of infection in amphibians. Infection also negatively affected the survival of developing amphibians. Mechanistically, eutrophication promoted amphibian disease through two distinctive pathways: by increasing the density of infected snail hosts and by enhancing per-snail production of infectious parasites. Given forecasted increases in global eutrophication, amphibian extinctions, and similarities between *Ribeiroia* and important human and wildlife pathogens, our results have broad epidemiological and ecological significance.

amphibian decline | emerging disease | environmental change

Emerging infections of humans and wildlife are often closely associated with anthropogenic alterations of the ecological and evolutionary relationships between hosts and pathogens, including climate change, biological invasions, land use change, and pollution (1–5). Owing to the complexity of pathogen–host–environment interactions, however, experimental evidence linking environmental change and increased infection is often lacking, leaving the ecosystem drivers of many emerging diseases unknown (5–7). In aquatic ecosystems, one of the most profound forms of ecological change is eutrophication, which is caused by anthropogenic inputs of nitrogen (N) and/or phosphorus (P) associated with agriculture, livestock, erosion, sewage waste, and atmospheric deposition (8–10). Because N and P often limit primary production, their addition causes marked shifts in ecosystem conditions (8, 9, 11).

Ecological theory (12) and limited field studies (see refs. 13 and 14) suggest that, unlike many environmental stressors, eutrophication will broadly enhance infection and the pathology of human and wildlife parasites. Nevertheless, experimental evidence linking nutrient enrichment and parasitism is largely absent, and the mechanisms through which eutrophication affects disease emergence remain poorly understood (12–15). Considering forecasted increases in global agricultural production and fertilizer application (10), as well as the persistence of anthropogenic P in agricultural soils and aquatic ecosystems (16), eutrophication will almost certainly become an increasingly severe problem in the coming century (12). This underscores the importance of understanding the mechanisms linking eutrophication and host–pathogen interactions and of balancing nutrient-mediated agricultural gains with concurrent increases in disease risk (11, 14).

Here we evaluated how elevated nutrient inputs leading to eutrophication affected the transmission and pathology of *Ribeiroia ondatrae*, a trematode parasite implicated in recent outbreaks of severe limb deformities in North American amphibians. This multihost parasite, which sequentially infects freshwater snails, larval amphibians, and waterbirds [see supporting information (SI) Fig. 4], has been causally linked to high frequencies of malformations (10–90%) in amphibian populations, including missing limbs, extra limbs, and malformed limbs (2, 17–21) (Fig. 1). Furthermore, infection and the resulting deformities substantially reduce amphibian survival, potentially contributing to widespread population declines and extirpations. However, the ecological drivers of parasite abundance, and the reasons for the apparent increase in amphibian deformities, have remained controversial (2, 19).

We experimentally tested the hypothesis that eutrophication enhances *Ribeiroia* transmission and identified the mechanisms responsible for this phenomenon. Uniquely, our experiment explicitly examines how nutrient enrichment affects transmission of a disease-causing parasite among multiple hosts within a complex life cycle. We hypothesized that, by increasing algal production, eutrophication would promote parasite infection through two, potentially complementary mechanisms. First, higher resource availability will increase the population growth of susceptible snail hosts (22), leading to enhanced parasite transmission and a higher density of infected snails (12). Second, higher resource levels will reduce infected snail mortality, increase snail body size, and enhance host vigor (15, 23–25), promoting parasite secondary production within infected individuals. Thus, a higher density of infected snails and a greater per-snail production of parasites should jointly drive an increase in amphibian infection and disease risk.

Results

Nutrient additions significantly enhanced all primary response variables (Figs. 2 and 3). Except where otherwise noted, values were $\log_{10}$-transformed before analyses. Eutrophication pro-

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The authors declare no conflict of interest.

Abbreviations: chl *a*, chlorophyll *a*; RM-ANOVA, repeated-measures ANOVA.

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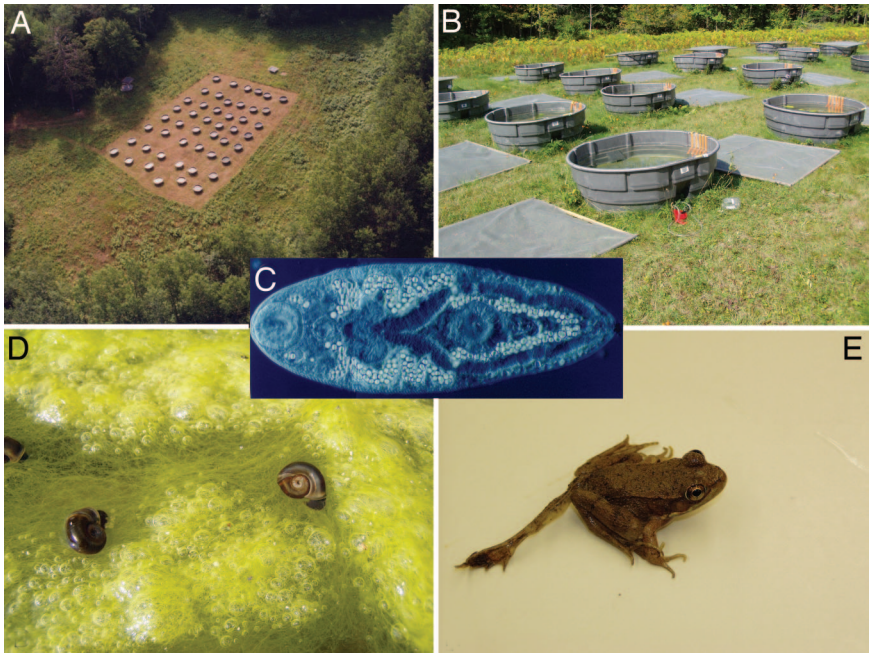

Fig. 1. Experimental setup. Bird's eye (*A*) and local (*B*) views of outdoor mesocosms used to investigate effects of nutrient enrichment on host–parasite interactions. Nutrients (N and P) and trematode eggs were added to mesocosms in a factorial experiment to understand how anthropogenic eutrophication influenced transmission of a multihost pathogen. The pathogenic trematode *R. ondatrae* (*C*, excysted metacercariae) uses pulmonate snails (*D*) as first intermediate hosts and amphibians as second intermediate hosts. Nutrient enrichment was hypothesized to promote algal growth, leading to an increase in the density and biomass of herbivorous snail hosts, thereby enhancing parasite transmission into snails. Infected snails with high resource availability were also expected to produce more parasite cercariae, increasing the risk of amphibian infection and pathology. In amphibians, *Ribeiroia* infection induces severe limb malformations (*E*) by disturbing the developing limb field.

moted growth of periphytic algae [measured as chlorophyll *a* (chl *a*)], which exhibited a monotonic increase in high nutrient mesocosms during the experiment (Fig. 2*A*). We also found significant effects for time and the time-by-nutrient interaction on phytoplankton chl *a* [repeated-measures ANOVA (RM-ANOVA), time: Greenhouse–Geisser adjusted $F_{[3.397,101.9]} = 6.832$, $P < 0.0001$; time-by-nutrients: Greenhouse–Geisser adjusted $F_{[3.397,101.9]} = 3.052$, $P = 0.027$]. However, nutrients had a significant between-subjects effect only on periphyton chl *a* ($F_{[1,30]} = 75.930$, $P < 0.0001$), reflecting the consistently stronger influence of nutrient enrichment on periphyton relative to phytoplankton.

Increases in periphytic algae led to enhanced egg production and a greater population biomass of herbivorous snails, regardless of parasite input level (Fig. 2 *B* and *C*). Total snail biomass increased by nearly 50% in the high-nutrient condition but declined by 10% among low-nutrient mesocosms (Fig. 2*C*). Results were comparable if we used mean snail density rather than biomass. Correspondingly, snail egg masses in high-nutrient mesocosms had, on average, twice as many eggs as those in the low-nutrient condition, highlighting the enhanced fecundity of snails in this condition (mixed model, nutrients: $F_{[1,56.17]} = 15.88$, $P < 0.0001$; mean eggs per egg mass ± 1 SE: high nutrients, 24.8 ± 1.3, low nutrients, 11.8 ± 1.5; $n = 115$). Parasite input level did not affect snail biomass (RM-ANOVA, $F_{[2,30]} = 0.009$, $P = 0.60$), snail egg production ($F_{[2,30]} = 0.001$, $P = 0.99$), or chl *a* levels ($F_{[2,30]} < 1.772$, $P > 0.18$).

Nutrient level and *Ribeiroia* egg input jointly determined the density of infected snails among treatments (Fig. 3 *A* and *B*). Increases in parasite egg input caused an increase in infected snail density; however, this effect was significantly enhanced by nutrient addition (Fig. 3 *A* and *B*). Comparable results were achieved if we used the total number of cercariae produced per sampling date or the prevalence of infection (arcsin square-root-transformed) among snails >10 mm as response variables (see SI Fig. 5). To evaluate the effect of eutrophication on cercarial production among actively shedding snails, we conducted a mixed-model analysis with nutrient status and parasite input level as fixed factors, snail size as a covariate, and cercarial production as the response variable. Snail subjects were nested within mesocosms and within sampling date. Eutrophication significantly enhanced per capita production of cercariae (Fig. 3*C*), whereas parasite input and the parasite-by-nutrient interaction had no detectable effects. Pooling across dates and parasite conditions, infected snails from high-nutrient mesocosms produced, on average, twice as many cercariae as snails from low-nutrient mesocosms (Fig. 3*C*). Infected snail size was also a significant covariate in predicting cercarial production (Fig. 3*C*), suggesting that nutrient-mediated increases in host size facilitated increases in parasite production.

Ribeiroia egg input level and nutrient status each positively affected infection abundance in amphibians, which ranged from 0 to 48 metacercariae ($n = 338$) (Fig. 3*D*). Owing to increases in infected snail density and in per-snail release of cercariae, larval amphibians in eutrophic treatments exhibited a 2- to 5-fold increase in infection relative to amphibians in low-nutrient mesocosms. Because green frogs normally require >1 yr to metamorphose at this latitude, limb development among most animals was insufficient to evaluate malformation status. Nevertheless, these infection levels are well within the range of values known to induce limb malformations in amphibians (17). Nutrient status also enhanced amphibian size (total length, ANOVA, $F_{[1,28]} = 10.728$, $P = 0.004$), whereas parasite treatment negatively affected amphibian survival (arcsin square-root-transformed) (ANOVA, $F_{[2,15]} = 4.247$, $P = 0.03$) (see SI Fig. 6).

Discussion

Ecologists and epidemiologists are increasingly challenged to understand how large-scale agents of environmental change

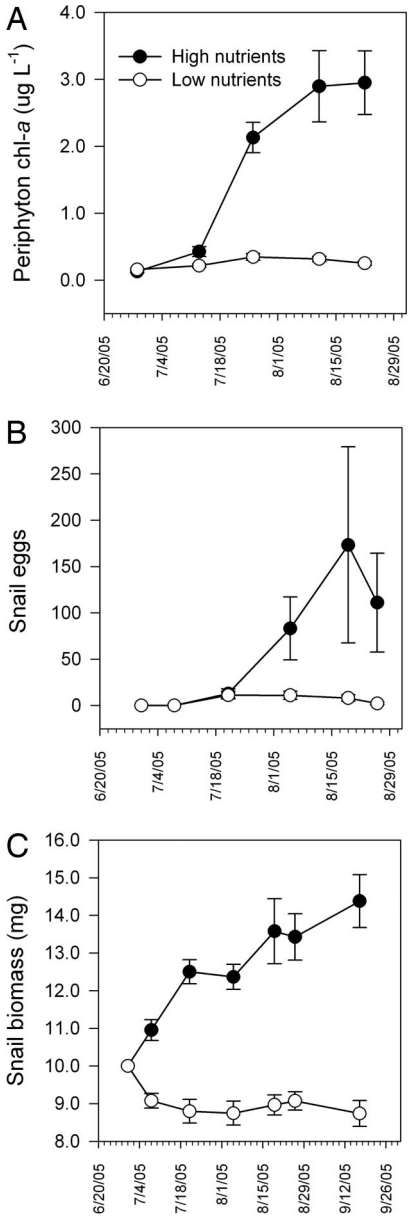

Fig. 2. Effects of nutrient enrichment on algal and snail growth. Nutrient enrichment significantly enhanced periphyton chl *a* (*A*) (RM-ANOVA, nutrients: $F_{[1,30]} = 75.93$, $P < 0.0001$; time × nutrients: Greenhouse–Geisser corrected $F_{[2.061,120]} = 23.747$, $P < 0.0001$), snail egg production (*B*) (RM-ANOVA, nutrient status: $F_{[1,30]} = 9.803$, $P = 0.004$; time × nutrients: Greenhouse–Geisser adjusted $F_{[2.58,77.39]} = 6.92$, $P = 0.001$), and dry mass of the snail host population (*P. trivolvis*) (*C*) (RM-ANOVA, nutrients: $F_{[1,30]} = 68.46$, $P < 0.0001$; time × nutrients: Greenhouse–Geisser corrected $F_{[2.96,\ 88.88]} = 21.802$, $P < 0.0001$). Snail density was converted to total dry mass by using the following equation (mass in grams = 0.0002 × [length in mm]$^{2.7232}$; $R^2 = 0.96$). Values are mean ± 1 SE and are pooled among parasite egg treatments, because parasite level did not significantly affect measured response variables.

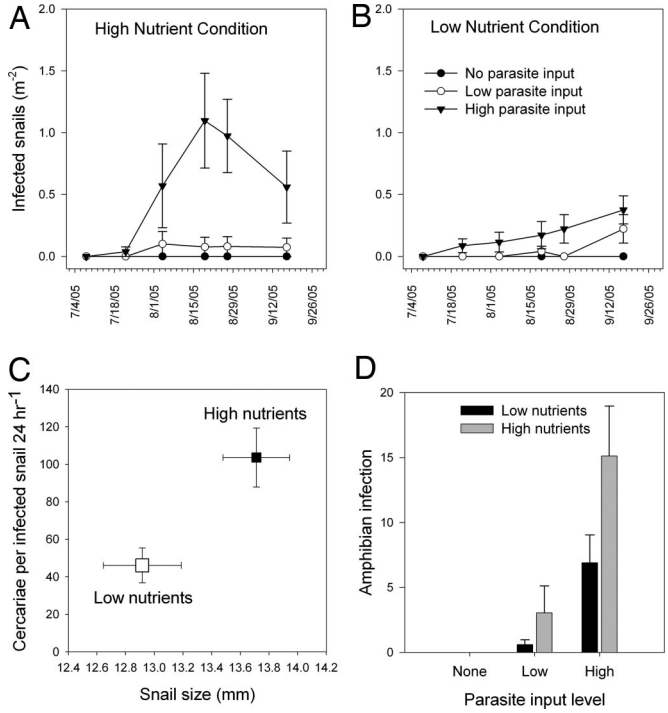

Fig. 3. Influence of eutrophication on *Ribeiroia* infection in snails and amphibians. Effects of *Ribeiroia* egg input level on the density of infected snail hosts (*P. trivolvis*) under high-nutrient conditions (*A*) and under low-nutrient conditions (*B*) (parasite input: $F_{[2,30]} = 18.917$, $P < 0.0001$; nutrients: $F_{[1,30]} = 11.079$, $P = 0.001$; parasite × nutrients: $F_{[2,30]} = 8.368$, $P = 0.001$). (*C*) Influence of nutrient condition and snail size ($\log_{10}$-transformed) on the per capita production of cercariae by infected snails (with snails nested within mesocosm and date sampled; mixed-model analysis, nutrients: $F_{[1,31.477]} = 5.20$, $P = 0.03$; snail size: $F_{[1,87.09]} = 28.604$, $P < 0.0001$). (*D*) Mean abundance of *Ribeiroia* metacercariae within larval green frogs as a function of *Ribeiroia* egg input level and nutrient condition (ANOVA, parasite input: $F_{[2,28]} = 19.27$, $P < 0.0001$; nutrient input: $F_{[1,28]} = 5.289$, $P = 0.02$). No metacercariae were recovered from amphibians in the "no-parasite" treatment. Values are mean ± 1 SE.

affect host–pathogen interactions (1, 4–7). Our study is the first to experimentally link aquatic eutrophication and transmission of a multihost parasite. In freshwater ecosystems, eutrophication is a widespread and growing problem with sharply negative effects on water quality, but the indirect effects of nutrient pollution on human and wildlife diseases are largely unexplored (8, 9, 11–14). Parasite-induced malformations in amphibians, which may have increased in prevalence and severity in recent decades (17–21), cause elevated mortality and morbidity in affected populations, but heretofore the environmental drivers of increased infection were largely unknown (but see ref. 2). By explicitly manipulating the inputs of nutrients and parasite eggs into experimental mesocosms, our results emphasize the importance of interactions among eutrophication, host dynamics, and parasite transmission. Importantly, the effects of eutrophication not only increased infection among first intermediate hosts (snails), but cascaded through the parasite's life cycle to increase amphibian infection, thereby elevating the risk of mortality and malformation.

Eutrophication promoted parasite infection and amphibian disease risk through two related but distinct mechanisms. First, increases in primary production resulting from nutrient addition enhanced the growth, reproduction, and survival of herbivorous snails, increasing the availability of first intermediate hosts for *Ribeiroia* miracidia (Fig. 2). In turn, this led to an increase in the density and prevalence of infected snails (Fig. 3 *A* and *B*). Second, eutrophication caused infected snails to nearly double their individual production of cercariae relative to infected snails in low-nutrient mesocosms (Fig. 3*C*). This likely occurred because of enhanced snail survival and growth under high-resource conditions (older and larger snails generally produce more parasites) or because of an increased capacity to translate snail resources into parasite secondary production (12, 15, 23–25). Importantly, although nutrient-mediated increases in infected host size contributed strongly to the observed increase in cer-

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carial production, nutrient input level also had direct positive effects on cercarial release, suggesting that our results reflect changes in both host size and parasite productivity (26). Taken together, the increase in infected snails and in the *per capita* production of cercariae led to significant increases in *Ribeiroia* metacercariae among co-occurring amphibian larvae (Fig. 3*D*). Considering that most theoretical models of trematode parasites assume equivalent production of cercariae among infected hosts regardless of environmental conditions, these results have important implications for understanding how environmental change indirectly affects pathogen transmission. Although the long developmental period of *Rana clamitans* ($\approx$1 yr) precluded inclusion of limb malformations as a response variable, the strong relationship between *Ribeiroia* infection and malformation frequency in amphibians (2, 17–21) suggests that the observed increase in infection can reasonably be expected to increase malformation likelihood.

In nature, the relationship between eutrophication and parasitism is likely to be variable and more complex than reported here, but several lines of evidence suggest that the proposed linkage between nutrient enrichment and *Ribeiroia* extends beyond the results of our experiment: (*i*) Available field data support correlations among eutrophication, snail host biomass, and *Ribeiroia* infection (19–21). (*ii*) Definitive hosts (especially birds) are often attracted to eutrophic environments, thereby increasing the input of parasite eggs deposited through feces (13, 26). In our experiment, nutrient enrichment and parasite inputs were independent variables, effectively decoupling this positive feedback. If definitive hosts are also more likely to consume infected amphibians as a result of their malformations (17), this will further amplify the effect of eutrophication on *Ribeiroia* abundance. However, definitive host abundance and activity will also depend strongly on the availability of suitable habitat, suggesting that the effects of eutrophication on infection will be context-dependent (27). Eutrophic wetlands embedded within environments favorable to bird hosts (e.g., rangeland), for example, may be more likely to support *Ribeiroia* and malformation epidemics than similarly eutrophic wetlands surrounded by less hospitable bird habitat (e.g., row crop agriculture). (*iii*) Finally, although our experiment included a simplified aquatic community, limited evidence suggests that inclusion of higher trophic levels, including snail and amphibian predators, may enhance infection through shifts in host behavior and life history strategies. By achieving size refugia, planorbid snails, such as those used by *Ribeiroia*, can achieve competitive dominance in high-nutrient environments (22), whereas amphibian larvae exposed to chemical cues from predators will often reduce their activity, thereby increasing their risk of trematode parasite infection (28). Nevertheless, given the inherent complexity and variability of natural communities, further study is needed to understand under what conditions eutrophication is likely to enhance infection and pathology. The current experiment included only two levels of nutrients (ambient vs. enriched), leaving open the question of whether the response of parasitism to eutrophication is linear or more complex, limiting the extent to which these results can be extrapolated over a broader range of nutrient levels.

An increase in *Ribeiroia* infection and malformations owing to progressive eutrophication could pose a serious risk to affected amphibian populations. *Ribeiroia* is a pathogenic parasite, causing direct and indirect (e.g., via malformations) mortality in amphibians (e.g., ref. 16 and this study). Although our experiment was short-term, sustained increases in the levels of *Ribeiroia* infection may precipitate decline or collapse of the amphibian population over longer time scales. In natural environments, the overwintering of infected snails may compound the effects of eutrophication among years. Indeed, by the end of our experiment, the number of prepatent (immature) snails infected with *Ribeiroia* more than doubled the total number of infected snails, forecasting substantially higher densities of infected snails for the following year. Because *Ribeiroia* causes reproductive castration in infected snails (21), high levels of infection may eventually reduce the snail population, possibly leading to cyclical patterns in *Ribeiroia* abundance and amphibian malformations. Moreover, eutrophic habitats frequently have high levels of contaminants such as pesticides or heavy metals, which may compromise the immune resistance of amphibians and further increase their susceptibility to parasite infection (2, 29), provided that such contaminants do not adversely affect the abundance of infected snail or bird hosts. Thus, as natural wetlands continue to be altered or destroyed, amphibians, now the most imperiled class of vertebrates worldwide (30), may be increasingly forced to use marginal, often eutrophic habitats that may be hotspots for disease.

Our results have broad applicability to other multihost parasites and their hosts. Recent increases in a variety of human and wildlife multihost parasites have been linked to eutrophication, including cholera, salmonid whirling disease, West Nile virus, coral diseases, and malaria (13, 14, 31–33). Trematode parasites similar to *Ribeiroia* that use snails as intermediate hosts also infect humans, ranging from the nuisance, but relatively innocuous, cercarial dermatitis to the pathogenic schistosomiasis, which is estimated to afflict 200 million people across Africa and Asia (35). If the life cycles of *Schistosoma* spp. are similarly affected by eutrophication, forecasted increases in agricultural nutrient applications in developing countries where schistosomiasis is endemic could hinder or inhibit efforts to control this disease. Ultimately, parasites that use multiple hosts through their life cycles are embedded in food webs with many connections to environmental drivers such as climate change, nutrient mobilization, and biotic exchange (1, 5, 6, 36). Such large-scale drivers can have substantial impacts on parasites and their hosts, with consequences for plant, animal, and human health. By understanding these impacts we will be better able to forecast disease risk in a changing world.

Methods

Experimental Design. To evaluate how eutrophication affected *Ribeiroia* transmission among hosts throughout its life cycle, we conducted a 2×3 factorial experiment manipulating the inputs of nutrients (ambient and elevated) and parasite eggs (none, low, and high) in mesocosms containing a community of pulmonate snails, larval amphibians, zooplankton, and algae (Fig. 1). Experiments were conducted in 1,200-liter mesocosms established outdoors near the University of Wisconsin Trout Lake Station (Fig. 1). Mesocosms were randomly assigned to condition and replicated six times for a total of 36 mesocosms. We initially seeded mesocosms with 1,000 liters of lake water, 22 kg of commercial "play sand" as substrate, and 30 g of $CaCO_3$ to promote snail shell growth. To provide inocula of algae and zooplankton, we added 50 ml of lake sediment and 30 ml of concentrated zooplankton and phytoplankton from each of five local wetlands.

We stocked mesocosms with 50 randomly selected uninfected snails (*Planorbella trivolvis*) collected and pooled from three local wetlands (mean size $\pm$ 1 SE = 12.8 $\pm$ 0.15 mm). To avoid inadvertent introduction of infected snails into the experiment, we isolated all snails individually into 50-ml vials for 24 h and subsequently examined the associated water for trematode cercariae. We dissected an additional 200 snails to ensure that prepatent (immature) infections of any trematode were rare ($<$0.5%) and that *Ribeiroia* was completely absent. We collected green frog (*R. clamitans*) egg masses ($n = 3$) from three wetlands in northern Wisconsin and allowed them to hatch and develop in the laboratory [stage 26 (37)] before adding 75 randomly selected individuals to each mesocosm on July 20, 2005. Meso-

cosms were covered with 1-mm mesh lids to minimize colonization of unintended flora and fauna.

Experimental Manipulation: Nutrient and Parasite Additions. We experimentally enhanced N and P in half of the mesocosms by adding 4.7 g of NH_4NO_3 and 0.4 ml of 85% H_3PO_4, respectively, and left remaining mesocosms at ambient nutrient concentrations. These one-time additions were selected to achieve initial concentrations of ≈1,800 μg·liter^{-1} of N and 200 μg·liter^{-1} of P for 945 liters of water (20:1 molar ratio). One week later, after algae had begun to use nutrient resources, average total unfiltered concentrations of N and P in the high- and low-nutrient treatments were 873 ± 31.4 μg·liter^{-1} N and 101.8 ± 3.1 μg·liter^{-1} P (high nutrients) and 467.3 ± 2.1 μg·liter^{-1} N and 10.3 ± 0.4 μg·liter^{-1} P (low nutrients), respectively. These levels are well within the range of values observed in natural amphibian habitats (19, 20). A recent survey of amphibian habitats in Wisconsin found nutrient concentrations of 2,859 μg·liter^{-1} N and 348 μg·liter^{-1} P for agricultural (eutrophic) wetlands and 1,235 μg·liter^{-1} N and 43 μg·liter^{-1} P for forested wetlands (R.B.H., unpublished data).

To obtain embryonated eggs of *Ribeiroia*, we experimentally infected laboratory rats (n = 10) with 50 metacercariae isolated from infected amphibians. After 2 weeks, rat fecal matter was collected on wet paper towels, soaked in spring water for 24 h, filtered through a sieve series, and incubated in the dark at 28°C for 3 weeks (38). Water was aerated continuously and changed weekly. We estimated *Ribeiroia* egg density ± 1 SE by counting the numbers of embryonated eggs in five 20-μl aliquots examined at ×200 magnification. We added eggs to mesocosms biweekly at one of three levels: 0 (control), 150 ± 11 (low egg input), and 1,500 ± 111 (high egg input). Eggs were placed into 1-liter plastic chambers suspended at the mesocosm surface and allowed to hatch and subsequently seek out susceptible snail hosts. We used suspended chambers to ensure that eggs were exposed to sunlight, which stimulates hatching, and equipped chambers with four openings covered by a 1-mm mesh screen to allow hatching parasite miracidia to escape the chamber and enter the mesocosm. After hatching, parasite miracidia were allowed to locate and infect susceptible snail hosts, which, after a period of intrasnail maturation, developed into parasite rediae. Rediae, in turn, produced mobile cercariae that emerged nightly from infected snails and actively infected larval amphibians, wherein they formed metacercariae. For mesocosms in the "no-parasite" treatment, we added a comparable volume (0.02–0.2 ml) of sieved and incubated feces from uninfected rats to equalize any nutrient additions administered through fecal material.

Sampling Schedule and Data Collection. Biweekly throughout the summer, we quantified levels of algal growth, snail density and reproduction, snail infection prevalence, and the daily, per-snail release of cercariae. In this manner we evaluated the effects of eutrophication on *Ribeiroia* transmission into snails (miracidia to rediae) and from snails into amphibians (cercariae to metacercariae) (see SI Fig. 4). We collected phytoplankton chl *a* from open water samples (2 liters) and filtered it onto 45-μm glass fiber filters, whereas we isolated periphyton chl *a* from 5 × 2.8-cm strips of flagging tape suspended on the sides of each mesocosm. Filtered samples were frozen for 24 h, extracted in methanol, homogenized, and centrifuged. Chlorophyll values were measured on a spectrophotometer. Predominantly, we were interested in the effects of nutrient addition on periphyton rather than phytoplankton growth, because the former provides an important food resource for herbivorous snails. Moreover, we expected that, within our experiments, phytoplankton standing stock would be controlled by zooplankton grazing, rather than by nutrient limitation.

Over this same time period, we visually enumerated the numbers of snails, snail egg masses, and eggs per egg mass within each mesocosm. To avoid unintentional bias in data collection, mesocosms were numerically coded without any indication of treatment condition. We classified snails into four size categories (1–5 mm, 5.1–10 mm, 10.1–15 mm, and >15 mm) that could consistently be classified visually (>95% observer accuracy) and determined the number of eggs per snail egg mass by counting eggs from up to a maximum of 10 randomly selected egg masses on 11 × 17-cm Plexiglas sheets placed within each mesocosm. Beginning on July 7, 2005, we estimated snail infection prevalence and daily cercarial production by randomly selecting 25 adult snails (>10 mm) from each mesocosm and isolating them individually into 50-ml centrifuge tubes allowed to float overnight within mesocosms. Released cercariae were identified and enumerated to provide estimates of snail infection prevalence and per capita production of *Ribeiroia* cercariae. All snails were returned to mesocosms the following morning. In total, 5,342 snails were examined for cercarial release, of which 94 were confirmed infected with *Ribeiroia*. Infected snails released an average ± 1 SE of 83.6 ± 11.2 (range: 2–651).

Winter conditions did not allow us to maintain the experiment between years, and we destructively sampled the mesocosms on September 15. We dissected all snails >5 mm that did not release cercariae to determine the number of prepatent (immature) infections and measured (total length in millimeters) all surviving amphibian larvae. We necropsied 10 larvae from each mesocosm (including individuals from the "no-parasite" treatments) to quantify *Ribeiroia* metacercariae.

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SEMIOCHEMICALS, TRAPS/TARGETS AND MASS TRAPPING TECHNOLOGY FOR MOSQUITO MANAGEMENT

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ABSTRACT. Technologies which utilize semiochemicals, traps/targets and mass trapping are relatively new for management of adult mosquito populations. To date most of the emphasis has been on developing barriers of attractant-baited and insecticide-impregnated targets. The most successful continuous use of this type of technology has been at Stevens' Landing, Collier County, FL. Recently, commercially available traps have been evaluated for their ability to reduce nuisance populations of mosquitoes. Use of Mosquito Magnet™ Pro traps along a nature trail on an isolated island (Atsena Otie) in the Gulf of Mexico resulted in a significant reduction in annoyance caused by the black salt marsh mosquito *Ochlerotatus taeniorhynchus* (Wiedemann). This chapter presents a brief discussion of semiochemicals (behavior modifying) and mosquito traps, strategies for using them for insect control, and an overview of the recent advancements in research activities conducted to evaluate using combinations of semiochemicals and mass trapping techniques for adult mosquito management.

INTRODUCTION

Traditionally, mosquito control in the United States has relied largely on the use of chemical insecticides for population management. This is especially true for the control of adult mosquito populations. This strict reliance on chemical insecticides has recently been a cause of concern. During the past 2 decades there has been a sharp rise in the public's apprehension about the use of chemical insecticides. This apprehension combined with other emerging realities (development of resistance in mosquito populations to chemical insecticides, the fact that none of the mosquito adulticides commonly used were developed recently, and that mosquito control is a niche market) has made consideration of alternative control technologies to chemical insecticides an area of active research. Resistance places the availability of chemical insecticides for mosquito control at risk. Availability of new insecticides for mosquito control is also threatened by the fact that as pesticide companies merge to form multinational conglomerates, the most profitable markets are those that drive corporate decisions (e.g., agricultural and pest control markets). Decisions to develop new pesticides will be determined by the financial bottom line. At present it may require $50 million or more to develop and register a new pesticide with the U.S. Environmental Protection Agency (USEPA). Several years of a new pesticide's patent life are likely to elapse before costs are recouped and profits accrue (Rose 2001). Mosquito insecticides, especially adulticides, are used at very low rates of active ingredient per acre that, although minimizing human and environmental risk, limit sales volumes and margins. Some seasons have few mosquitoes, so the limited sales are even lower. Therefore, compared to agricultural and structural pest control uses, companies may not perceive insecticides for mosquito control as very profitable. Product liability also plays an important role in reducing incentives because of possible personal and class-action lawsuits or court injunctions against pesticides being applied over populated areas (Rose 2001).

Therefore to preserve our existing arsenal of chemical insecticides, there has been an increased emphasis over the past 20 years in developing integrated pest management (IPM) programs that include surveillance, source reduction, larvicides, and biological control, as well as public education (Rose 2001). In order for these IPM programs to be effective, development of new technologies needs to be encouraged. One new technology that is under active investigation is the use of semiochemical baited traps/targets for mass trapping adult mosquitoes.

Interest in exploring this technology for mosquito control was stimulated in 1989 when 25 scientists from the United States, Canada, the United Kingdom, and the Netherlands met in Minneapolis, MN, to participate in the first International Symposium on Hematophagous Insect Attractants, sponsored by the Metropolitan Mosquito Control District. The consensus of this group of scientists was that semiochemicals and possibly targets and traps could play a significant role in the control and surveillance of hematophagous insects. The extent of that role was the subject of much discussion (Kline 1994). The group's optimism was based on examples from agricultural entomology and the success that tsetse fly workers achieved with attractant-baited, insecticide-impregnated targets in Zimbabwe (Vale

1993) and elsewhere throughout Africa. Until the mid-1980s tsetse control in Zimbabwe was achieved by the broadcast of endosulfan or deltamethrin from the air or the application of DDT from the ground (Alsopp 1984). The steadily successful use of targets led to the complete replacement of aerial spraying in 1991 (Vale 1993).

The potential use of semiochemicals and innovative trapping technology in the detection, monitoring, and control of economically important insect species has been pioneered by agricultural entomologists (Jacobson 1972, Marx 1973, Mitchell 1975, Roelofs 1979). These pioneers recognized that semiochemicals play an important role in the behavior and communication of insects, but unlike conventional insecticides are not toxic or detrimental to either the target species or any other organism. It was quickly discovered that another major advantage of these behavior-modifying chemicals is that they are generally very specific and can thus be used to selectively target the insect pest or vector of importance.

This paper will provide a brief background on semiochemicals and basic strategies for utilizing them for control, and then summarize some recent activities conducted to explore the feasibility of using combinations of semiochemicals and mass trapping techniques for adult mosquito management.

BRIEF SEMIOCHEMICAL DEFINITIONS

Semiochemicals are signaling chemicals produced by one organism that incite changes in the behavior or physiology of another organism. These chemicals can be classified by whether they influence members of the same species as that of the producer (i.e., *intra*-specific signals), or influence a different species than that of the producer (i.e., *inter*-specific signals). The former are classified as pheromones, whereas the latter can be divided into kairomones and allomones, depending on whether the response in the recipient is favorable to the recipient or to the producer of the signal, respectively (Shorey 1977). Another way to categorize semiochemicals is in terms of the type of response they induce in the recipient: stimulant, arrestant, attractant, or repellent, depending on whether they cause increase or decrease in a certain behavioral activity, or orientation towards or away from the source of the chemical, respectively (Shorey 1977).

While examining semiochemical use for insect control, researchers have focused on those semiochemicals that elicit a response towards the source, hence the often-used term attractants to identify these compounds (Shorey 1977). These include chemicals used 1) to locate a mating partner (sex pheromones), 2) conspecifics (aggregation pheromones), 3) a suitable host or food source (feeding stimulants, plant volatiles, and animal odors), or 4) an oviposition site (oviposition pheromones and attractants). Although this chapter will focus on the use of such chemical attractants in the suppression of insect pests and vector species, the importance of non-chemical attractants, such as visual attractants, is acknowledged. The combination of chemical, visual and physical attractants should be an integral part of any control strategy.

GENERAL APPROACH AND TECHNIQUES FOR THE USE OF SEMIOCHEMICALS IN INSECT CONTROL

Mosquito ecologists can learn much on the use of semiochemicals for population management by reading the agricultural entomology literature. One of the pioneers in the utilization of semiochemicals for insect population management, Shorey (1977) has pointed out that it is essential to acquire a detailed knowledge of the insect's own normal use of semiochemicals in order to devise an efficient system to use these chemicals for the management of insect pests and vectors by chemically modifying their behavior to our advantage. As previously mentioned, these chemicals do not kill insects directly, but rather affect the behavior of individuals in some manner that reduces their survival rate. The basic steps that are usually involved in the development of a system that utilizes attractants are (modified after Roelofs 1979):

1. Chemically characterize the attractive chemical(s) and document potency by laboratory and field tests. In case of a mixture of attractants, their optimum component ratio needs to be established.
2. Deermine the attractancy of the chemical(s) by comparison to natural source of the attractant (e.g., live insect, host), or by the percentage of catch or mating disruption obtained with a known population.
3. Address the optimum release rate, advantages, and disadvantages of various formulations or trap design in which the chemicals will be incorporated in the field.
4. Conduct small field tests to evaluate height, spacing, and placement patterns of the release points to determine optimum values for these parameters. In cases where the attractant(s) will be combined with a toxicant, sterilant, or pathogen, the number of bait centers that is needed to significantly reduce the insect population has to be determined.
5. Use large-scale field trials to evaluate the efficacy of the system. It is important to document the duration and spatial scale of the experiment, climatic conditions during the test, as well as population density of the targeted species, and how efficacy was

determined (e.g., damage to crop, landing rates on humans, etc.).

BASIC METHODS FOR UTILIZING ATTRACTANTS (SEMIOCHEMICALS) FOR INSECT CONTROL

Three basic methods have emerged over the last few decades in which attractive semiochemicals are used to control insect pests and vectors, depending on the type of semiochemical utilized in the system. Nevertheless, all 3 approaches are aimed at causing a decline in the insect population, either by removing a large majority of individuals from the population (mass trapping), by having a large majority of females go unmated (mating disruption), or by using the attractant(s) to bring a majority of individuals into contact with a toxicant, sterilant, or pathogen (attracticide) (Roelofs 1979, Kydonieus and Beroza 1982, Day and Sjogren 1994).

Mass Trapping

Trapping systems for insects are important components in IPM programs. Trapping data are generally used to make decisions on the initiation or termination of control measures, as well as to assess efficacy of control approaches that have been implemented (Kydonieus and Beroza 1982). Given the enormous reproductive potential of insects, it is generally believed that large-scale mass trapping systems are only effective against low population densities, or when initiated early in the season to remove enough insects to limit the natural build-up of the population. Major difficulties in mass trapping are the large number of traps usually required, and the high cost of deploying and maintaining them (Kydonieus and Beroza 1982). Despite these acknowledged limitations, mass trapping has been the method most explored for mosquito population reduction to date.

Mating Disruption

In agricultural entomology, perhaps the most promising means of controlling insects with attractants has been the use of sex pheromones to disrupt the ability of an insect to locate a mating partner and therefore prevent them from propagating (Mitchell 1975, Carde and Minks 1995). Mating disruption is achieved by permeating the atmosphere with sex pheromone, although the precise mechanisms of how disruption works are still largely unknown (Mitchell 1975, Saunders 1997). Atmospheric permeation is accomplished by either aerial or ground applications of sex pheromone using sprayable formulations and various kinds of dispensers, respectively (see Mitchell 1975). Saunders (1997) discusses some of the factors affecting the success of mating disruption as a control technique. He mentions that efficacy depends on population density, food preferences of the target species, and the degree of isolation of the treated areas. Other important attributes of the target species include characteristics of mating behavior, pheromone release rates, and male responsiveness, including both the range of concentrations and the length of time during the day to which the males respond.

The technique is not confined to the disruption of mating by sex pheromones alone. There are also examples where the use of pheromones that inhibit the aggregation response of conspecifics has enjoyed some success and promise as a future control strategy. This approach, rather than mating disruption, seems to have the most promise for mosquito control. Mating disruption is not likely to be very important in managing adult mosquito populations because no potent sex pheromone has yet been identified for any mosquito species, but some of the principles and dispensing technology might be useful in developing a strategy for releasing behavior inhibitors (spatial repellents) to prevent adult female mosquitoes from finding their hosts (Kline et al. 2003).

Attracticides (Lure and Kill)

The third approach that has been investigated is to combine attractants with a killing agent, a concept that is often referred to as attracticide. In this method the attractant is used to lure insects to a device or area where they will be killed. There are variations on the attracticide theme, with different mechanisms of population suppression depending on the mortality agent, which can be a toxin (e.g., Steiner et al. 1970, Norval et al. 1996, Suckling and Brockerhoff, 1999), sterilant (e.g., Takken et al. 1986), or pathogen (e.g., Shapas et al. 1977). Another variation of this approach is the use of trap crops where insects are killed with an insecticide applied to the plants (Kydonieus and Beroza 1982). For mosquitoes a similar approach might be application of insecticide to resting sites or vegetated areas baited with attractants. The most commonly practiced method is the combination of attractant with an insecticide. Although still relying on insecticides, it has the advantage of greatly reducing the amount of insecticide applied, allows the choice of when and where the treatments will be made, and greatly reduces the impact on non-target organisms (Day and Sjogren 1994).

THE TSETSE STORY: IMPLICATIONS FOR MOSQUITOES

While there are an increasing number of success stories using semiochemical-based trapping technologies to control agricultural pests (Hardie and Minks 1999), the example that is most germane to this paper is the tsetse fly story. This is the most successful example of a mass-trapping program directed against a hematophagous insect. Removal trapping of tsetse flies has been successful in

various parts of Africa (Vale 1993, Torr 1994). Tsetse flies are responsible for the transmission of trypanosomiasis to humans (sleeping sickness) and domestic cattle (nagana) in large parts of sub-Saharan Africa. Much of the success of these removal trapping programs has been attributed to both the biological peculiarities of tsetse and a better understanding of tsetse behavior, which resulted in the development of effective targets that combine visual and olfactory attractants used by tsetse to locate their hosts (Vale 1993, Jordan 1995). The vulnerability of tsetse flies to trapping-out systems stems from their unusual life cycle. Compared to other insects, tsetse have an extremely low intrinsic rate of population increase (Hargrove 1988). Calculations based on life table data have shown that it is only necessary to catch some 1–4% of the female population per day in order to achieve effective control (Weidhaas and Haile 1978). Visual and olfactory attractants for *Glossina* spp. have been incorporated into several control trials and operations in a variety of ways. They can simply be used to improve the effectiveness of a trap, but more usually they are associated with a trap or target that has been treated with an insecticide (Jordan 1995).

Initial attempts of removal trapping of west African tsetse flies involved the manual collection and killing of tsetse attracted to human and visual attractive traps, respectively (Morris and Morris 1949). Populations of *G. palpalis* and *G. tachinoides* could be eliminated from small groves in 8–12 weeks through manual capture, but such areas were easily repopulated once captures ceased. Traps rapidly reduced the local tsetse populations by as much as 70% during the first few weeks of trapping and, when deployed at high densities, could neutralize the effect of immigration and result in a sustained reduction of tsetse flies in the area. Nevertheless, total elimination of tsetse in the study area could not be achieved. More recently, Laveissiere and Couret (1981) located 850 simple blue cloth targets (120 × 90 cm), each impregnated with deltamethrin, along 79 km of riverine habitat in the Ivory Coast to control *Glossina* spp. populations. They reported a 98% reduction of *G. tachinoides* and *G. palpalis* populations within a few days. Biconical traps, insecticide-impregnated blue cotton targets, and the sterile insect technique (SIT) were successfully integrated to eradicate *G. palpalis palpalis* in the center of a 1500 km^2 area of central Nigeria and the targets deployed at the boundary habitats proved to be effective barriers to reinvasion (Takken et al. 1986).

The control of tsetse species in other parts of Africa became more efficient with the discovery of odor attractants, such as carbon dioxide, acetone (Vale 1980), octenol (Hall et al. 1984), and phenols (Bursell et al. 1988). The first Zimbabwe trial was conducted on a small island in Lake Kariba, where tsetse were eradicated by using a combination of traps, baited with carbon dioxide and acetone, followed by deployment of insecticide-impregnated targets baited with acetone and octenol (Vale et al. 1986). In a subsequent program, some 2,500 deltamethrin-impregnated targets, consisting of black cloth baited with octenol and acetone, were deployed over an area of 600 km^2, resulting in a target density of 3–5 km^2. Within a 9-month period, populations of *G. pallidipes* and *G. morsitans morsitans* had declined by at least 99.99% in the center of the study area and their numbers reduced by more than 95% at the margins (Vale et al. 1988). Vale (1993) points out that odor-baited targets were then [in 1993] being used in Zimbabwe and have been successfully used to suppress or eradicate regional tsetse populations in other African countries, with little technical change, as in Zambia against *G. morsitans centralis* (Willemse 1991), or with certain modifications, as in Kenya against *G. pallidipes* (Dransfield et al. 1990).

The main implication for developing similar technology for mosquito population management is that control of tsetse by mass-trapping technology was not an overnight success story. Success was the result of a long, dynamic process achieved by the collaboration of many dedicated scientists from a variety of disciplines with a lot of government and private agency support (Kline 1994, Torr 1994). Development of mass trapping technologies for mosquitoes will not be achieved overnight either, but will also require close interdisciplinary collaboration among entomologists (with ecological and behavioral backgrounds), chemists, electrophysiologists and molecular biologists. The challenges to develop this technology for mosquitoes may be greater than for tsetse, especially in the United States. Most members of the mosquito control community are skeptical that mass trapping technology can work against mosquitoes. There are many more pest species and the intrinsic rate of population increase is high for most mosquito species. There is little financial support from government and private agencies, and therefore the critical mass of scientists working to develop this technology for mosquitoes has not yet materialized.

MOSQUITO TRAP/TARGET DEVELOPMENT

Four additional symposia and 3 workshops have been held on developing this technology for mosquitoes since the 1989 Minnesota symposium. The consensus of participants has been that the greatest priority for this technology to have any chance of success against mosquitoes is that more efficient and economical traps/targets and attractants need to be developed.

In 1989 2 basic types of mosquito traps were available for use: the Centers for Disease Control

(CDC) miniature light trap (Sudia and Chamberlain 1962) and the standard New Jersey (NJ) light trap (Mulhern 1942). Variations of these 2 trap types that could be purchased (Service 1993) differed widely in size, weight and electric power requirements, and type and intensity of light. One modification of the CDC was designed to reduce the capture of non-target insects by reversing the direction of air flow. This change sucked insects into a collection container above the trap, which discriminated in favor of mosquitoes and similar lightweight specimens. This modification, known as the updraft trap, had been demonstrated to increase the capture of some mosquito species (Rupp and Jobbins 1969, Wilton and Fay 1972). These traps were designed for routine surveillance, and were not intended for mosquito control. Trap collections for some species were increased by adding carbon dioxide (Rudolfs 1922), 1-octen-3-ol (octenol) (Takken and Kline 1989), and/or lactic acid (Kline et al. 1990).

In 1995 2 private companies initiated programs to develop traps specifically designed for mosquito control. BioSensory, Inc. (Willamantic, CT) developed the Dragonfly™, which used CO_2, octenol and heat as attractants. The CO_2 was released from compressed gas cylinders. Mosquitoes were killed by electrocution. Although this trap was made available to residential users, it was mainly developed for commercial applications and has never been marketed through retail outlets. In contrast, American Biophysics Corporation (North Kingston, RI) developed a series of traps known as Mosquito Magnets™ for both commercial and residential use. These traps also used CO_2 (generated by the combustion of propane), octenol and heat as the main attractants. In some models a thermoelectric generator was used to generate electricity to power the fans. This made the traps more portable and allowed them to be placed where most needed without reliance on either mains electricity or a portable generator.

After West Nile virus (WNV) invaded the United States in 1999, public concern for mosquito control increased. At this point many companies decided to develop mosquito traps to sell to residential users. A plethora of traps has since been developed and marketed. These traps come in a huge variety of designs, attractant combinations, and capture technologies. Most of the commonly available traps use CO_2 as the main attractant, which is generated either by the combustion of propane or supplied from cylinders of compressed gas (Table 1). Relatively few comparative efficacy studies of these traps have been conducted. Many have not even been tested in a scientific manner to determine their ability to capture mosquitoes or suitability for inclusion in any type of organized mosquito control activity.

MASS TRAPPING STUDIES AGAINST MOSQUITOES

Few studies have been conducted to evaluate mass trapping technology against mosquitoes of any species. A 3-year (1993–1995) project was conducted on Key Island, Collier County, FL (Kline and Lemire 1998) to evaluate the impact of a protective barrier around a designated resort area on attacks by pest species of mosquitoes, primarily *Anopheles atropos* Dyar and Knab, *Culex nigripalpus* Theobald, and *Ochlerotatus taeniorhynchus* (Wiedemann). The efficacy of single line barriers, consisting of either 52 traps (1994) or collapsible lambda-cyalothrin-impregnated targets (1995), baited with CO_2 (200 cc/min) + octenol (ca. 4 mg/h), spaced ca. 16.5 m apart, was evaluated with baited surveillance traps (model 512 CDC type trap, John Hock Co., Gainesville, FL, without a light source) on both sides of the barrier. Targets were suspended so the bottom was ca. 15 cm above ground level. The octenol and CO_2 were dispensed ca. 67 cm above ground level at ca. 4 mg/h over a 24-h period (octenol) and CO_2 at 200 ml/min. This combination of attractants and release rates was based on studies previously conducted with Everglades mosquito species (Kline et al. 1990, Kline et al. 1991b, Takken and Kline 1989). The traps were hung from metal poles so that the top of the trap was ca 1.8 m above ground level. Traps and targets performed equally well. Though not statistically significant ($p > 0.05$) there was a noticeable reduction in mosquito abundance in the resort area when the barrier was functional.

Collier Mosquito Control District (CMCD) continued this work in 1996. Instead of each target being supplied with CO_2 from individual tanks at each target location, the tanks were connected through a manifold to a control panel, which maintained a constant flow to the targets. Octenol was released near the area of the target where the CO_2 was released. The targets were sprayed with insecticide (either permethrin or lambda-cyalothrin) on a scheduled basis. The CMCD has performed several studies with this design of attractant-baited barrier line, or modifications of it. Each study was conducted to test various target designs, target spacing, or other parameters in order to develop the most efficient system (Stivers 2005).

This system was first evaluated in 1996 as a continuation of the studies conducted by Kline and Lemire (1998). A perimeter CO_2 pipeline was placed around a designated area of Key Island. Targets were placed along the pipeline and treated with insecticide every 2 weeks. Carbon dioxide was released through the targets at a rate of 200 ml/min. CDC surveillance traps were used to collect mosquitoes in order to determine the

Table 1. Some of the more commonly known commercial traps available for mosquito control.

Manufacturer	Trap Name	Attractants	Fan Power	Cost
	Utilizes Propane Combustion			
American Biophysics	MM-Defender	CO_2 Heat, Water Vapor, Octenol, Lurex	Mains electricity	$295
	MM-Liberty	Same as Defender	Mains electricity	$495
	MM-Liberty Plus	Same as Defender	Thermoelectric (cordless)	$695
	MM-Pro	Same as Defender	Thermoelectric (cordless)	$895
	MM-Solar Pro	Same as Defender	Thermoelectric (cordless)	$1195
	MM-Solar Pro Plus	Same as Defender	Thermoelectric (cordless)	$1395
Blue Rhino	Skeeter Vac (SV) -15	CO_2, Heat, Water Vapor, Octenol	Thermoelectric (cordless)	$200
	SV-27	CO_2, Heat, Water Vapor Octenol, Light Emitting Diodes (LEDs)	Thermoelectric (cordless)	$300
	SV-35	Same as SV-27	Thermoelectric (cordless)	$330
	SV-2000	Same as SV-27	Thermoelectric (cordless)	$350
Coleman	Deleto 1.5 (MD-2200)	CO_2, Heat, Water Vapor, Octenol	No fan	$179
	MD-2500	Same as MD-2200	Thermoelectric (cordless)	$329
Flowtron	Power Trap Utilizes compressed gas cylinders	CO_2, Heat, Water Vapor	Mains electricity	$399
EnviroSafe	MegaCatch Ultra	CO_2, Heat, UV Light, LEDs, Octenol	Mains electricity/12 V battery	$499
???	Mosquito Terminator	CO_2, skin emulator, UV and white light	Mains electricity/12 V battery	$450
BioSensory	Dragonfly	CO_2, Heat, Octenol		
	Utilizes no CO_2 as an attractant			
TekQuest	Mosquito Catcher	Heat, Blue LED	Mains electricity	$140
Flowtron	Galaxie Power-Vac	Heat, UV Light	Mains electricity	$150
Scotco Products	Mosquito Free	Heat, UV Light, LEDs, Mosquito FREE Brew (lactic acid base)	Mains electricity	$99
ISCA	Excalibur	Formulated pheromone attractant (Lurex 440), Heat	Mains electricity	$80
????	Bug Eater	UV light	Mains electricity	$99
EnviroSafe	MegaCatch Premier	Heat, UV Light, LEDs, Octenol	Mains electricity/12 V battery	$249

efficacy of the system. The data showed significant differences in the ratio of mosquitoes collected inside and outside of the line when the system was on compared to when it was off. However, the level of control that was achieved was considered to be insufficient for operational use (Stivers 2005).

In 1998, the CMCD began a study on the feasibility of using their attractant-baited barrier line technology at a condominium complex on Marco Island, FL, called Stevens' Landing. This site was surrounded by mangrove swamp that produced enormous broods of *Oc. taeniorhynchus*. The goal of this study was to determine whether the barrier line could be effective in protecting a populated area from adult mosquitoes. The system design was very similar to the design used on Key Island, with ca. 2400 ft of pipeline around the entire community and CDC traps to determine the number and species of mosquitoes inside and outside the line (Stivers 2005). This study was conducted over 3 years, testing variables such as target spacing, target shape, rate of CO_2 released, and octenol release method. The most efficient target spacing was 20 ft. The most effective target was "bucket" shaped, composed of regular weed block cloth hanging from the lid of a 5-gal bucket and hung from a metal pipe. This target was either dipped in or sprayed with insecticide (lambda-cyalothrin) bi-weekly for control purposes. During the final year of the study, liquid octenol was replaced with "stick" octenol to facilitate handling and reduce costs (Stivers 2005). The 3 years of data show that the line provided the residents with relief from mosquitoes. Statistical analysis demonstrated that there were fewer mosquitoes inside the barrier line when the system was on than when the system was off. The residents also noted the difference in mosquito abundance when the line was on or off. The system worked so well that the community at Stevens' Landing voted to have it installed permanently.

During the winter of 2001, the CMCD, in collaboration with Stevens' Landing, made the barrier line a permanent facility at the condominium. The system was upgraded burying the CO_2 pipelines, retrofitting the control panel with copper rather than plastic tubing, and replacing the 100-lb compressed CO_2 tanks with cryogenic carbon dioxide held in 500-lb tanks. The tanks were replaced weekly in order to provide the system with enough gas to attract mosquitoes. The CMCD purchased the materials for the pipeline and the targets and installed the entire system. The condominium association, however, was responsible for the costs of maintenance and operation. The permanent attractant-baited barrier line has been running at Stevens' Landing since the summer of 2001. The District no longer needs to aerially apply insecticide to the area, reducing the potential impact on non-target organisms in the mangrove environment surrounding the community (Stivers 2005).

While this control method has proven effective at Stevens' Landing, it is not a method that can be applied in all circumstances. Further testing is necessary to determine if the system can be equally effective in other areas.

MASS TRAPPING USING COMMERCIAL TRAPS

From August 2002 through October 2004 (Kline, unpublished data) a mass trapping experiment utilizing the Mosquito Magnet™ Pro (MM-Pro) was conducted on a group of 3 small isolated islands, collectively known as Atsena Otie, located in the Gulf of Mexico ca. 1 mi off the coast of Cedar Key, FL. Atsena Otie is managed by personnel from the Lower Suwannee Wildlife Refuge (LSWR). The study was initiated at the request of the LSWR manager who wanted mosquitoes controlled on the islands, but did not want pesticides to be used. Unfortunately, there was no historical baseline of mosquito trap collections for the island that could be used as a point of reference. But ample anecdotal evidence was provided by the LSWR rangers and local residents, who consistently and frequently stated that from May through October the islands are unsuitable for human visitations. Shortly after the salt marsh areas surrounding the islands are flooded, the islands become inundated with enormous populations of *Oc. taeniorhynchus*. Visitation is difficult even when covered with repellent and protective clothing. This indeed was the situation that was encountered in August 2002 when the traps for this study were being set up. Individuals setting up the traps wore pants, long sleeve shirts and headnets, all sprayed with Deep Woods OFF™ (SC Johnson, Racine, WI). Any exposed skin was also treated with this repellent. As a measure of mosquito activity, one pant leg was pulled up to the knee. It took less than 15 seconds for the mosquitoes to cover all available exposed skin from knee to ankle.

The MM-Pro was selected as the "treatment" trap based on its performance in large cage trap efficacy studies conducted against laboratory reared *Oc. taeniorhynchus* (Table 2). In these studies the MM-Pro caught 1.6× as many *Oc. taeniorhynchus* as its nearest competitor and resulted in the lowest landing/biting counts. Also this trap is completely portable since it uses a thermoelectric generator to produce electricity to operate its suction fans. This was an important feature since no electricity was available on Atsena Otie. This was a true test of the trap manufacturer's (American Biophysics, Inc.) claim that the MM-Pro will clear out a 1-acre area in 2 wk.

The island selected for the trap study was ca. 23 acres of land surrounded by salt marsh. A nature

Table 2. Relative trap efficacy determined in large outdoor cages against the black salt marsh mosquito *Ochlerotatus taeniorhynchus*.

Trap Type	% Captured[1]		Cost ($US)
	Collection Device	Landing/Biting Rate	
Mosquito Magnet (MM)-Pro	73.4	1.5	1295
MegaCatch	46.4	1.9	800
Dragonfly (UK model)	42.8	3.5	620
MM-Liberty	41.7	3.0	495
Mosquito Trap	37.5	4.4	399
Dragonfly (Biosensory model)	28.9	5.9	645
Deleto 1.0	27.8	13.4	199
Sonic Web	26.5	11.9	299
CDC (John Hock Model 512)	24.6	7.9	244
Power Trap	18.7	6.4	399
No Trap	—	19.8	—

[1] Based on 1000 4–6 day-old females released into a 30′ wide × 60′ long × 16′ high, gabled to 18′ screened cage 2 h before sunset; landing/biting counts made 2 h after sunrise on day after release.

trail nearly bisected this island. Either 21 (2002 and 2003) or 22 (2004) MM-Pro traps were placed along this trail such that each trap covered a radius of ca. 1 acre. In 2002 and 2003 modified MM-Pro traps (Figure 1) were used because the collection nets of the unmodified trap filled up too quickly. Therefore, the traps were modified so that the mosquitoes were collected into an 11.5-liter container instead of the standard net. By 2004 (16 months) population pressure on the island was reduced to a level that unmodified traps could be used. In addition to the CO_2 and water vapor generated by the combustion of propane, each trap was also baited with octenol.

Fig. 1. Modified Mosquito Magnet™ Pro trap with 11.5-liter collection container used on Atsena Otie in 2002 and 2003.

Two MM-X traps, located 1/3 and 2/3 of the way along the trail were used as surveillance traps. These traps were baited with octenol and 500 ml/min CO_2 released from a 20-lb compressed gas tank. One similarly baited MM-X trap was placed on each of the 2 adjacent "untreated" islands. In 2002 and 2003, 2 additional MM-X traps were placed on the mainland in the LSWR as a means of comparison. In 2004, 5 MM-X traps were scattered on the mainland throughout the city of Cedar Key. Each year the surveillance traps were operated for at least 2 wk before and after the treatment traps were turned on. In 2002 the treatment traps were operated from August 23 through October 4; in 2003 from May 20 through October 14; and from June 12 through October 19 in 2004.

In 2002 biting pressure was significantly reduced within 2 wk. In contrast to the pretreatment scenario where an exposed leg was completely covered with viciously biting mosquitoes from knee to ankle in 15 seconds, no repellent was needed. From this point on, trap collections were made in shorts and short sleeve shirts. There were occasional blips of biting activity within a week after larval developmental sites were flooded either by rainfall events or by tides. Usually these blips would last for only 1 or 2 days. Basically, this scenario was repeated each year after the traps had been operating for 2 wk. Each year the biting pressure progressively declined after each flooding event. This led to speculation that perhaps the "egg bank" was being depleted due to the enormous number of potential first time egg layers being removed from the island's population. Mosquito immigration and emigration were unknown factors. The data are still being analyzed, but preliminary analyses indicate that by the end of the third year there

appeared excellent (80–90% population reduction), sustained control despite the fact that the area was hit by multiple hurricanes.

Additional mass trapping studies were conducted in residential areas of Gainesville, FL, during the 2003 and 2004 mosquito seasons in a collaborative study between the U.S. Dept. of Agriculture, Center for Medical, Agricultural and Veterinary Entomology (CMAVE) research laboratory and the Gainesville Mosquito Abatement program (Gainesville, FL) (Kline, unpublished data). These studies were conducted to evaluate the efficacy of this technology in a non-isolated area, where mosquito diversity was high (>33 species) and the areas to be protected were surrounded by numerous larval developmental sites. Two separate neighborhoods were surrounded by 12 unmodified MM-Pro traps. Each trap used octenol as an additional attractant. CDC traps and landing rate counts were utilized twice a week to monitor mosquito species composition and abundance within the perimeters of these "treated areas" compared to similar "untreated" (not surrounded by MM-Pro traps) residential areas. Preliminary analysis indicates moderate control, ca. 50% reduction in captures by the CDC monitoring traps in "treated" compared to "untreated" residential neighborhoods. Similar unpublished studies using MM-Pro traps in residential areas have been conducted in St. Augustine, FL (Ruide Xue, personal communication). Residents in "treated" areas in both Gainesville and St. Augustine perceived the traps to be effective at reducing mosquito abundance in their yards.

In another residential trapping study, the Salt Lake City Mosquito Abatement District used the MM-Pro in an attempt to give residents some relief from *Oc. sierrensis*, a tree hole mosquito species (Hougaard and Dickson 1999). These mosquitoes are very aggressive which makes them a neighborhood nuisance. The species stays close to their source area, which makes removal trapping a viable control method. The MM-Pro traps were used at several locations over the course of the summer in 1999. When contacted by telephone, homeowners responded that the MM-Pros had really helped and that the mosquitoes were under control. Abatement personnel concluded that the MM-Pro was an effective tool in helping to control this species of tree hole mosquito. They stated that it by no means got rid of all of the mosquitoes, but it did give people some relief and peace of mind that the abatement district was trying to help.

CONCLUSIONS

Mass trapping using semiochemical baited traps for mosquito population management is a relatively new technology. A summary of the tsetse mass trapping effort illustrates that the technology can be successful against a hematophagous pest. Despite the tsetse fly success story, many, if not most, medical entomologists, mosquito scientists and control personnel are skeptical that it can be effective against adult mosquitoes. In contrast the technology is finding an increasingly wider acceptance among agricultural entomologists.

The tsetse story illustrates that much basic research into the target species' biology, behavior and ecology is a prerequisite for success. It also demonstrates that a multidisciplinary effort is required. The few mosquito studies that have been conducted and presented in this paper indicate that this technology has promise for mosquito population management, but the challenges to success appear greater than for controlling tsetse. Mosquitoes are more prolific than tsetse and most travel greater distances. There are also many more nuisance mosquito species in any given geographic area than occurs with tsetse.

The mosquito studies presented in this paper illustrate some of the promises and some of the problems encountered so far. Both the attractant-baited targets and MM-Pro studies indicated promise for managing populations like *Oc. taeniorhynchus*, which is very attracted to the combination of attractants used (CO_2, octenol, heat and water vapor) in these studies. The more attractants used (e.g., MM-Pro traps used all types of attractants compared to targets which did not use heat and water vapor), the better the population reduction. The technology worked best on the isolated islands where one species (*Oc. taeniorhynchus*) was clearly the dominant species compared to mainland residential areas like Gainesville where many important nuisance species of different genera were present. Thus, the Gainesville study demonstrates that "one size does not fit all." Different attractant combinations, delivery systems, and trap types may be required to attract and effectively capture different populations.

Another scenario in which the technology looks promising is in areas like Salt Lake City, UT, where a single dominant nuisance species (*Oc. sierrensis*), stays in backyards close to the larval developmental sites (tree holes).

So far, studies utilizing mass trapping technology against adult mosquitoes have been conducted on a limited spatial and temporal scale. They have not progressed beyond small-scale trials. Before large-scale mosquito trials are conducted it is essential that we acquire a better understanding of the spatial distribution of target species, their dispersal patterns, and their population dynamics in general. Studies with simulation models of tsetse (Hargrove 1988) and mosquitoes (Ritchie and Montague 1995) have shown that population dynamics are most sensitive to dispersal and adult mortality parameters. The dispersal capacity of targeted mosquito

species can have a great influence on the potential of attractant-baited trapping systems. A high dispersal rate, as is present in *Oc. taeniorhynchus* (Provost 1957), could impose problems in attempts to reduce local populations, as the risk of reinvasion would be high. In the small-scale tests with *Oc. taeniorhynchus* on both Key Island and Atsena Otie, whenever a flooding event occurred in the larval developmental sites, several days later the perimeter of targets or array of MM-Pro traps would be overwhelmed (but only temporarily). The technique might have greater potential against mosquitoes with a relatively small dispersal activity, such as *Cx. nigripalpus* (Nayar et al. 1980) or *Oc. sierrensis* (Hougaard and Dickson 1999).

Knowledge of the spatial distribution of targeted species is also necessary as it relates to finding an optimal placement strategy for traps or targets for maximum impact on targeted species (Kline 1998). Questions that need to be answered include the impact of location, spacing, and height of deployed traps or targets on the effectiveness of the system. Day and Sjogren (1994) describe 4 approaches to the deployment of traps or targets: 1) to attract mosquitoes away from where protection is desired; 2) to situate traps around the protection area as a perimeter barrier; 3) to place traps/targets individually within the protection area; and 4) to intercept mosquitoes during dispersal from breeding sites or resting sites.

Knowledge of basic population parameters and dynamics is also essential to determine the degree of trapping required to attain a certain level of population control (Weidhaas and Haile 1978). These authors estimated that, depending on the biotic potential of the mosquito species, the trapping requirement could be as high as 40% per day to achieve a substantial reduction in the population. Service (1995) theorized that the immense biotic potential and population densities of mosquitoes make it unlikely that traps or targets alone could reduce mosquito populations to an acceptable level. This scenario is often encountered for certain floodwater mosquitoes, such as *Oc. taeniorhynchus* and *Ae. vexans*, which are characterized by rapid and large explosive population outbreaks. Yet the studies cited above reveal that in selected circumstances, control can be achieved with such species by attractant-baited traps or targets. Perhaps, attractant-baited traps could be utilized equally or more effectively in areas where mosquito densities remain relatively low or for species that breed in permanent water bodies and are often characterized by a more gradual population build up.

Acceptance by the public and/or the professional mosquito control community of new technologies, such as mass trapping and other biological control technologies will not be easy because they have grown accustomed to the immediate control obtained through spraying with chemical insecticides, despite recognizing the negative side effects. Nevertheless the use of semiochemical-baited traps/targets, either locally or on an area-wide scale, needs to be perfected to the level that it can be incorporated into selected IPM programs. Parks, resorts, golf courses, and other recreation areas may be good candidates for this technology. It is important to educate residents, decision-makers and the general public about both the potential and the constraints of attracting and trapping, e.g., a lower level of control may need to be accepted in certain circumstances. With the development of sufficiently effective traps and increased diversity of effective attractant combinations for different mosquito species, trapping systems could be used routinely in the future as behavioral control measures and could be added to the growing list of biologically based technologies for mosquito control.

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Evaluation of various models of propane-powered mosquito traps

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ABSTRACT: Large cage and field studies were conducted to determine the efficacy of various models of propane-powered mosquito traps. These traps utilized counterflow technology in conjunction with catalytic combustion to produce attractants (carbon dioxide, water vapor, and heat) and a thermoelectric generator that converted excess heat into electricity for stand-alone operation. The cage studies showed that large numbers of *Aedes aegypti* and *Ochlerotatus taeniorhynchus* were captured and that each progressive model resulted in increased trapping efficiency. In several field studies against natural populations of mosquitoes two different propane traps were compared against two other trap systems, the professional (PRO) and counterflow geometry (CFG) traps. In these studies the propane traps consistently caught more mosquitoes than the PRO trap and significantly fewer mosquitoes than the CFG traps. The difference in collection size between the CFG and propane traps was due mostly to *Anopheles crucians*. In spring 1997 the CFG trap captured 3.6X more *An. crucians* than the Portable Propane (PP) model and in spring 1998 it captured 6.3X more *An. crucians* than the Mosquito Magnet Beta-1 (MMB-1) trap. Both the PP and MMB-1 captured slightly more *Culex* spp. than the CFG trap. ***Journal of Vector Ecology* 27(1): 1-7. 2002.**

Keyword Index: Mosquito trap design, propane traps, trapping efficiency.

INTRODUCTION

Attractant-baited traps are being considered as an alternative to the use of chemical insecticides in certain situations for mosquito population management , and this has stimulated private industry to develop new trap types. Kline (1999) reported on the efficacy of one promising new trap developed by American Biophysics Corporation (ABC), (East Greenwich, RI), known as the Counterflow Geometry trap (CFG). The CFG trap is very efficient in attracting most species of mosquitoes. Its major drawbacks are that it requires an external source of power and mosquito attractants, such as heat and carbon dioxide (CO_2), must be provided. Therefore, ABC has been developing a series of propane-powered traps as an alternative option for mosquito management. These traps utilize the patented counterflow technology of the CFG trap to capture mosquitoes and catalytic combustion of propane to produce its own attractants, which include CO_2, heat and water vapor. A thermoelectric generator uses excess heat from the combustion process to generate electricity to run the traps' fans. The specific objectives of the present study were: (1) to determine if propane-powered traps could attract and capture laboratory-reared *Aedes aegypti* (Linnaeus) and *Ochlerotatus taeniorhynchus* (Wiedemann) released into an outdoor cage, and (2) to measure their capture efficacy compared with ABC's professional surveillance (PRO) and CFG traps against natural populations of woodland mosquito species.

MATERIALS AND METHODS

Traps Tested

Professional (PRO) trap. The PRO trap is a relatively new trap that is similar in design to the Centers for Disease Control (CDC) trap (Sudia and Chamberlain 1962). It is described in detail by Kline (1999) and McNelly (1995). The trap is powered by a 6-V, 10-ampere-hour rechargeable gel-cell battery (Figure 1A).

Counterflow Geometry (CFG) trap. This trap design utilizes a new mosquito capturing principle known as counterflow, first described by Kline (1999). In operation the trap utilizes 2 fans energized by a 12-V DC battery to provide counterflow at the trap entrance. A 40-mm fan (Delta model DFB0412M) creates a CO_2-enriched airflow plume from CO_2 supplied from a compressed gas cylinder, which exits vertically down a center pipe. An upflow is created by an 80-mm fan (Delta model DFB0812H) that causes any mosquito in the vicinity of the trap entrance with a flight speed less than ca. 3.5 m/sec to be entrained into the upflow and forced into the

trap interior. The CFG trap was hung from a pole so that the bottom of the attractant plume was ca. 50 cm above the ground (Figure 1B).

Propane-1. This trap utilizes the same trap body as the CFG trap, but its attractants (CO_2, heat and water vapor) are produced by external catalytic combustion of propane. Excess heat is utilized by an external thermoelectric generator to produce electricity to power the 2 fans (Figure 1C).

Portable-CO_2 (PC) Trap. This trap uses the same principle of counterflow technology as the CFG trap, but the counterflow is produced by a different trap design. The fans are contained within a durable metal housing. This trap is designed to compare the efficacy of CO_2 supplied from a compressed gas cylinder with that produced from catalytic combustion of propane. The fans are powered by 12-V DC gel cell batteries. This design also allows the use of the same amount of CO_2 as that supplied to the CFG and PRO traps (Figure 1D).

Portable Propane (PP). This trap has the same basic design as the PC trap, but utilizes a catalytic combustion unit to convert propane into CO_2, heat and water vapor. The trap uses the same metal housing construction for the fans and catalytic burner as the PC trap. The trap also incorporates a thermoelectric generator, which uses a portion of the heat to produce electricity to power the two fans. This self-generation of electricity allows the trap to be a totally stand-alone unit, which can run unattended for 20 days when a standard (9 kg) barbecue grill propane tank is used. This also allows placement of the trap where the mosquito problems are rather than where a convenient power supply may exist (Figure 1E).

Mosquito Magnet Beta-1(MMB-1) . This trap utilizes a more efficient catalytic converter. The housing unit was redesigned and constructed from a durable plastic. A clip was provided to attach ABC's standard 1-octen-3-ol (octenol) release pack. This trap was briefly marketed in 1999 as the Counterflow 2000™ (Figure 1F).

Mosquito Magnet Beta-2 (MMB-2). This trap was basically the same as the MMB-1 model, but the attractant release tube was modified to incorporate a chamber within the CO_2 outflow tube to entrain octenol in the attractant plume rather than having an octenol packet attached near the trap entrance.

Mosquito Magnet (MM). This version of the trap is commercially available. The power generator/converter has been modified for easy field service and higher reliability. This model has a more robust, molded plastic housing unit, but its basic design is similar in appearance to the MMB-1.

Nicosia Device. This trap is an experimental device (patent # 5,657,576) based on pulsed liquid technology that produces an acoustical signal, similar to that produced by the circulatory system of a host animal; the details of what attractant signals are produced are not completely understood. Although this trap attracts some mosquitoes without CO_2, the combination of acoustic signal and CO_2 is recommended for maximum effect. The trap utilizes an electrocution grid to kill the attracted mosquitoes. It is included in this study because it introduces a new technology that shows promise and will be refined as its mode of attraction is better understood.

Large Cage Studies

Evaluations of each model of propane trap were made in a large outdoor screened enclosure (9.2 m wide x 18.3 m long x 4.9 m high on the sides and 6.1 m high at the peak) where 1,000 3- to 4-d-old laboratory-reared *Oc. taeniorhynchus,* and on some nights an equal number of *Ae. aegypti,* were released ca. 2 h before sunset, 250 of each test species into each corner. Each release night the mosquitoes were marked with a different color of fluorescent powder so that they could be followed through time. Trap collections were retrieved approximately 90 min after sunrise and the number of mosquitoes caught in the traps was determined. Only the mosquitoes with the previous release night's marking were included in trap efficiency determinations.

The impact that test traps had on mosquito landing rates in the test cage was also determined. Landing counts were taken along a transect extending from the southwest (SW) to northeast (NE) corners of the test cage. Three sampling stations were established along this transect: the 2 corners (SW and NE) and the midpoint of the transect, which was where the test traps were located. For 6 min at each station, mosquitoes were aspirated into a collection tube with a modified portable vacuum cleaner as they attempted to land on the human host. A different collection tube was used at each station. The mosquitoes were killed with cold and counted.

The Propane-1 trap was evaluated for two nights (November 20-22, 1996). One thousand *Oc. taeniorhynchus* and *Ae. aegypti* were released each night. The PC trap was operated for four nights between December 17, 1996 and January 24, 1997. One thousand *Oc. taeniorhynchus* were released each night, but 1000 *Ae. aegypti* were available for release on only the first night (December 17-18, 1996). Initial studies of the PP trap were conducted for 10 nights from March 5 - May 6, 1997. *Oc. taeniorhynchus* were utilized all 10 nights, but *Ae. aegypti* were only available for 5 of these nights.

Sustained studies were conducted for 20 nights from September 21- November 15, 1997. *Oc. taeniorhnychus* were used each night; *Ae. aegypti* were available for 14 nights scattered throughout this testing period. Initial evaluations of the MMB-1 trap were conducted for 4 consecutive nights (April 28 through May 1, 1998). *Oc. taeniorhynchus* were available for testing each night, but *Ae. aegypti* were only available on the first night. Frequent evaluations were conducted from October 27 - December 11, 1998. During 21 nights of testing, *Oc. taeniorhynchus* were available each night, but *Ae. aegypti* were available only for 5 test nights. The MMB-2 trap became available for testing in May 1999. It was evaluated for 11 nights from May 25 - June 11, 1999. Both species were evaluated each night. The MM trap, a commercially produced model of the propane traps, became available for testing in late June 1999. It was evaluated for 9 nights between June 22 and July 14, 1999. *Aedes aegypti* was utilized each night and *Oc. taeniorhynchus* all nights except the first.

Field Studies

Several field trapping experiments were conducted against natural populations of mosquito species associated with wooded wetlands located near a water management area in Gainesville, FL. Three replications of a 4 x 4 Latin square experiment were conducted during March through April 1997. In this experiment four trap types were compared (PRO, CFG, PC and PP). In each replication the four trap types were alternated so that each trap type occupied each trap station for a single night. Trap stations were located 18 m apart. Carbon dioxide was supplied to the PRO, CFG and PC traps from 9 kg compressed gas cylinders. The flow rate used for all 3 three trap types was 500 ml/min. Control of CO_2 flow rate was achieved with FLOWSET1 (ABC, East Greenwich, RI) which is comprised of a pressure regulator with output fixed at 15 psig, a 10 micron line filter, a 500 ml/min flow control orifice, and quick-connect luer fittings. The CO_2 produced (350 ml/min) for the PP trap was by catalytic combustion of propane, which was determined by ABC to be ca. 350 ml/min. This combustion process also produced heat and water vapor. One package of OCT 1 Slow Release 1-Octen-3-ol (octenol with a release rate of ca. 0.5 mg/h) (ABC, East Greenwich, RI) was taped to each trap. A fresh package was used for each experiment.

Another 4 x 4 Latin square experiment was conducted at this woodland site with collection dates May 11-15, 1998. The four trap types used in this experiment were the PRO, CFG, Nicosia Device and the MMB-1. While the MMB-1 trap generated its own CO_2 through the catalytic combustion of propane, CO_2 was supplied to the PRO, CFG and Nicosia Device in the same manner as in the previous field experiments. An octenol package was taped to each trap.

Data for all field experiments were analyzed with Statistical Analysis System (SAS) PROC GLM and Means/REGWQ for the analysis of variance and means comparisons (SAS Institute 1985).

RESULTS

Large Cage Studies

In these studies, 19.8- 62.6% of *Ae. aegypti* and 26.1-71.2% of *Oc. taeniorhynchus* were captured by these propane-powered traps, and the larger the collection, the more the landing count was reduced (Table 1). These data, indicate with a few exceptions, that the progressive development of the propane-powered traps resulted in better trapping efficiency. This was especially true for *Oc. taeniorhynchus* where the first trap model (Propane-1) caught an average of 261 specimens over a 2 night period, and the most recent model tested in the cage (MM) caught an average of 712.3 specimens for 8 nights of evaluation. The exception to this was that the average catch for the PP model was 413.5 compared to 371 for the MMB-1 trap. The continuous improvement trend was not as clear for *Ae. aegypti* where the PP trap caught an average of 469.2 specimens compared to 378.5 and 378.3 for the MMB-1 and MMB-2 models, respectively. However, the latest model tested (MM) caught greater than 3X as many (626.1 vs 197.5) as the Propane-1, and 25% more specimens than the PP.

Field Studies

In the first replicate of the 1997 Latin square experiment, 4389 mosquitoes were collected. The CFG trap captured the most specimens (2320), followed by PP (834), PC (789), and PRO (446). Fifteen species of mosquitoes were collected. The number of mosquito species collected in each trap type was 13 for PC, 12 each for PP and CFG and 11 for PRO. Only 3 species had at least 100 total specimens caught. These were in order of decreasing abundance, *Anopheles crucians* Wiedemann (3015), *Ochlerotatus canadensis* (Theobald) (666), and *Culex salinarius* Coquillett (339). Eight species, *Oc. canadensis*, *Ochlerotatus dupreei* (Coquillett), *Ochlerotatus infirmatus* Dyar and Knab, *Ochlerotatus sticticus* (Meigen), *An. crucians*, *Coquillettidia perturbans* (Walker), *Culex erraticus* Dyar and Knab, and *Culex salinarius* (Coquillett), were collected in all four trap types. Three species, *Aedes vexans* (Meigen), *Anopheles quadrimaculatus*, and *Psorophora ferox* (Von Humboldt) had at least 1 specimen in 3 trap types. *Ae. vexans* was not collected

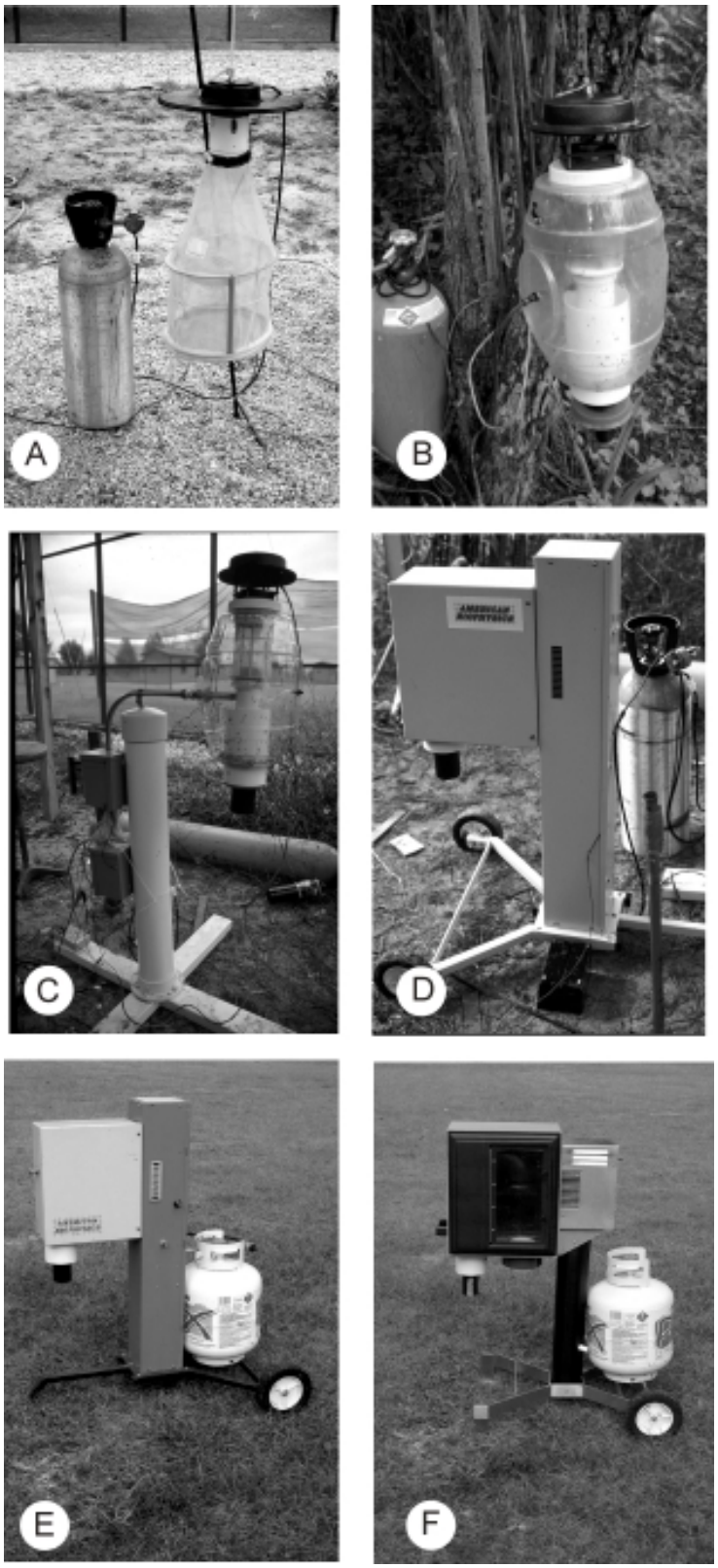

Figure 1. Trap types tested in either large cage and/or field studies: A. Professional; B. Counterflow Geometry; C. Propane-1; D. Portable Cylinder; E. Portable Propane; F. Mosquito Magnet Beta-1.

in the PP trap; *Anopheles quadrimaculatus* Say was not collected in the CFG trap; and *Ps. ferox* was not collected in the PRO trap. *Ochlerotatus mitchellae* (Dyar) was only collected in the PC and PP traps. *Anopheles punctipennis* (Say) was only collected in the PP and PRO traps. *Oc. triseriatus* (Say) was only collected in the PC trap. *Culiseta melanura* (Coquillett) was only collected in the CFG trap.

In the second 1997 replicate, 3164 mosquitoes were collected. The CFG trap again collected the most specimens (1343) and the PRO the least (119), but the PC trap collected more than the PP trap (1170 vs 532). Fifteen species were again collected. No *Oc. triseriatus*, *An. punctipennis* or *Cs. melanura* were collected, but a single specimen of three species not collected in the first replicate, *Ochlerotatus atlanticus* Dyar and Knab, *Aedes albopictus* (Skuse) and *Psorophora columbiae* (Dyar and Knab) was collected in the PP, CFG, and PRO traps, respectively. Two specimens of *Ps. ferox* were collected in the CFG trap. At least one specimen of *Oc. canadensis*, *An. crucians*, *Cq. perturbans*, *Cx. erraticus*, and *Cx. salinarius* was collected in all four trap types. *Ochlerotatus dupreei* and *An. quadrimaculatus* were collected in all trap types except the PRO. A single specimen of *Oc. mitchellae*, *Oc. sticticus*, and *Ae. vexans* was captured in both the CFG and PP traps. The CFG trap had the greatest species diversity (13), followed in decreasing order by the PP (12), PC (8), and PRO (7) traps. Five species were collected in sufficient numbers for further analyses. In order of decreasing abundance, these were *An. crucians* (2433), *Cq. perturbans* (301), *Cx. salinarius* (169), *Oc. canadensis* (113), and *Cx. erraticus* (98).

In the third 1997 replicate, a total of 2755 specimens was collected. The CFG caught the most (1613), followed in decreasing order by PC (552), PP (480), and the PRO (116). Sixteen species were captured. The CFG and PP each caught 13 different species; the PRO and PC each

Table1. Mean (±SD) responses of laboratory reared *Oc. taeniorhynchus* and *Ae. aegypti* to various traps in large outdoor cage studies.

	Species					
	Ochlerotatus taeniorhynchus			*Aedes aegypti*		
Trap Type	n	Landing Count	Net	n	Landing Count	Net
Propane-1	2	90.0 (48.1)	261.0 (166.9)	2	28.0 (39.6)	197.5 (82.7)
PC	4	123.0 (30.9)	314.2 (149.6)	1	0.0	229
PP	30	70.0 (63.6)	413.5 (192.9)	19	12.9 (14.7)	469.2 (226.3)
MMB-1	25	100.2 (71.1)	371.0 (234.4)	6	55.2 (53.4)	378.5 (232.1)
MM-1	11	38.3 (36.8)	520.1 (200.9)	11	11.0 (15.3)	378.3 (168.7)
MM-2	8	13.0 (8.4)	712.3 (141.4)	9	2.6 (3.5)	626.1 (198.0)

Table 2. Mean (±SD)[1] response of total mosquitoes collected and 5 most commonly collected species to various trap types located in a woodland wetland in Gainesville, FL, in Spring 1997.

	Trap types used			
Species	CFG	PP	PC	PRO
Total	422.6 (200.9) a	153.1 (174.5) bc	211.8 (174.5) b	56.4 (49.3) c
Ochlerotatus canadensis	40.9 (44.6) a	9.5 (10.5) b	13.3 (12.7) b	9.3 (11.0) b
Anopheles crucians	328.3 (156.9) a	91.4 (61.3) bc	173.3 (169.1) b	27.2 (27.0) c
Coquillettidia perturbans	21.3 (19.0) a	17.2 (16.2) a	12.8 (12.4) ab	5.1 (4.7) b
Culex erraticus	5.3 (6.2) a	5.7 (7.6) a	4.1 (5.9) a	1.1 (1.9) a
Culex salinarius	19.7 (12.7) ab	23.5 (16.3) a	5.2 (3.4) c	9.7 (13.7) bc

[1]n = 12 nights. Means in the same row followed by the same letter are not significantly ($P > 0.05$) different; Ryan-Einot-Gabriel-Welsh multiple range test (SAS Institute 1985).

Table 3. Mean (±SD)[1] response of total mosquitoes collected and 5 most commonly collected species to various trap types located in a woodland wetland in Gainesville, FL, in Spring 1998.

	Trap types used			
Species	CFG	MMB-1	NICOSIA	PRO
Total	653.3 (253.4) a	197.5 (86.9) b	148.0 (73.9) b	41.5 (13.2) b
Ochlerotatus canadensis	10.5 (5.1) a	6.5 (5.3) a	6.3 (3.5) a	4.3 (3.9) a
Anopheles crucians	496.8 (175.9) a	78.3 (63.9) b	50.3 (32.8) b	8.5 (5.5) b
Coquillettidia perturbans	83.0 (49.4) a	57.5 (10.4) ab	38.0 (25.4) ab	20.5 (9.0) b
Anopheles quadrimaculatus	4.3 (3.8) a	1.3 (1.3) a	0.8 (0.9) a	0.0 (0.0)a
Culex salinarius	48.0 (27.9) a	51.5 (16.3) a	42.3 (21.9) a	3.5 (3.1) b

[1]n = Four nights. Means in the same row followed by the same letter are not significantly ($P > 0.05$) different; Ryan-Einot-Gabriel-Welsh multiple range test (SAS Institute 1985).

caught 8 species. No specimens of either *Ae. albopictus* or *Ps. columbiae* were captured. At least one specimen of *Oc. canadensis*, *Oc. dupreei*, *An. crucians*, *Cq. perturbans*, *Cx. erraticus*, and *Cx. salinarius* were collected in all four trap types. *Ochlerotatus infirmatus* was collected in all trap types except the PC. *Oc. triseriatus* and *An. quadrimaculatus* were collected in all types except the PRO. *Oc. atlanticus* and *An. punctipennis* were only collected in the CFG and PP traps. *Ochlerotatus mitchellae* and *Ps. ferox* were only collected in the CFG trap. *Oc. sticticus* and *Ae. vexans* were only collected in the PP trap. *Cs. melanura* was only collected in the PRO trap.

In terms of total *Ae. canadensis* and *An. crucians* collected, the CFG collections were significantly different ($P < 0.05$) than the other trap collections (Table 2) for all 3 replicates combined. There were no significant differences among trap types for *Cx. erraticus.* There was no significant difference between the CFG and PP trap collections for *Cx. salinarius*, but both trap types caught significantly more than either the PC or PRO. The PP trap caught more specimens of both *Cx. salinarius* and *Cx. erraticus* than any of the other trap types. There was no significant difference ($p > 0.05$) between the CFG, PP, and PC in their *Cq. perturbans* collections, but the collections of this species by all 3 were significantly ($P < 0.05$) greater than the collections of the PRO trap.

In May 1998, seventeen species of mosquitoes were collected, 13 by the Nicosia Device, 12 by the CFG, and 9 each by the MMB-1 and PRO traps. Overall, sixteen males and 4161 females were captured during 4 nights of trapping. The 16 male specimens consisted of 3 *An. punctipennis* (1 in MMB-1 and 2 in PRO), 6 *Cq. perturbans* (3 in MMB-1 and 3 in CFG), and 7 *Cx. salinarius* (6 in MMB-1 and 1 in the Nicosia Device). The majority of female mosquitoes were collected in the CFG trap (2613) followed in decreasing order by the MMB-1 trap (790), Nicosia Device (592) and the PRO trap (166). Seven species (Oc. *canadensis*, *Oc. infirmatus*, *An. crucians*, *An. punctipennis*, *Cq. perturbans*, *Cx. erraticus*, and *Cx. salinarius*) were collected by all four trap types. Two species (*An. quadrimaculatus* and *Cs. melanura)* were collected by 3 trap types. *An. quadrimaculatus* was not caught by the PRO trap; *Cs. melanura* was not caught by the MMB-1 trap. *Ae. vexans* was collected only by the PRO and Nicosia Device. Only one specimen of the seven other species was collected by different trap types: *Ae. albopictus* by MMB-1; *Ae. aegypti*, *Oc. sticticus*, and *Oc. triseriatus* by CFG; Oc. *atlanticus*, *Orthopodomyia signifera* (Coquillett), and *Ps. columbiae* by the Nicosia device. Only 4 species had at least 100 specimens collected during the 4 nights and their response to the various trap types were statistically analyzed. In order of decreasing abundance they were *An. crucians* (2535 females), *Cq. perturbans* (6 males, 796 females), *Cx. salinarius* (7 males, 581 females), and *Oc. canadensis* (110 females). *An. quadrimaculatus* was also statistically analyzed.

These analyses showed that the total mosquito collection was significantly greater ($p < 0.05$) with the CFG trap than any of the other trap types (Table 3). In terms of total mosquitoes collected, none of the other trap types were statistically different from each other. Much of the difference between the CFG and MMB-1 traps was the large disparity in the number of *An. crucians* caught in each trap type. This was the only species in which there was a statistical difference between the CFG and the MMB-1. In fact, the CFG caught 6.3X more

An. crucians than the MMB-1. The MMB-1 caught slightly more *Cx. salinarius* than the CFG.

DISCUSSION

The data reported here indicate that propane-powered traps can capture up to 71.2% of host-seeking mosquitoes released into a confined space such as the test cage. They also collect a diversity of mosquito species under field conditions. In fact, the propane traps caught 17 of the 21 species caught in the field studies. The only trap that performed better was the CFG, which caught 19 different species. Neither the CFG nor the propane traps caught any *Or. signifera*, a species rarely caught in any trap, or *Ps. columbiae*, which was not abundant during these studies. In terms of the most abundant species, compared to the CFG, the propane traps did not do well in capturing *An. crucians*, but captured slightly more *Cx. erraticus* and *Cx. salinarius*. So these data do indicate that there may be a problem getting some species to respond in proportion to their abundance. This needs to be investigated further.

These studies were not designed to directly evaluate the potential of the propane traps for use as mosquito management tools, but do suggest that this may be a worthwhile endeavor. The field data show that the propane-powered traps collected 2.7X and 4.8X more mosquitoes in the spring 1997 and 1998 experiments, respectively, than the PRO traps. PRO traps are very similar in design and efficacy to CDC traps which, when baited with octenol and less CO_2 (200 ml/min compared to 350 ml/min for the propane traps), were used to cause significant reduction in heavy mosquito populations in Collier County, FL (Kline and Lemire 1998).

Some attributes which make this a viable option are that these traps produce their own basic attractants (CO_2, heat, and water vapor) and electricity. The trap's catalytic combustion process converts twenty pounds of propane into 60 pounds of CO_2, thus reducing the bulk of supplies to be carried to the field. This production of the basic attractants eliminates the need to find a commercial source of CO_2, either as a compressed gas or as dry ice, which can be a difficult task to achieve in many areas of the USA and other places in the world. When it can be obtained, the use of compressed gas from cylinders requires the use of expensive regulators and flow devices to control the CO_2 release rate and a rental fee is often required for the cylinders. Dry ice is often even more difficult to obtain than compressed gas. If the dry ice option is used, it needs to be resupplied to the traps that utilize this technique on a daily basis. Obtaining and storing the necessary quantities of dry ice can be a problem. Production of its own electricity means that the placement of the trap is not restricted to a source of main line electricity. It also means that batteries and battery chargers are not required. This portability means the traps can be strategically placed in remote areas where mosquito problems occur. These traps also do not require daily maintenance. They will operate continuously as long as the propane fuel supply lasts. Because propane is readily available almost everywhere in the world, these traps can be transported and used almost anywhere. Another favorable feature is that few nontarget organisms are collected, so these traps are environmentally friendly.

With the uses and availability of chemical insecticides likely to become more restricted and more mosquito species becoming resistant to the available insecticides, alternatives such as attractant-baited traps need to be evaluated for their control potential. Studies need to be initiated now before the insecticide options become even more limited. Although it will take time to develop more efficient traps, these data show a progressive improvement in the efficacy of the propane-powered traps.

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West Nile virus

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West Nile virus is a mosquito-borne flavivirus originally isolated in 1937 from the blood of a febrile woman in the West Nile province of Uganda. The virus is widely distributed in Africa, Europe, Australia, and Asia, and, since 1999, it has spread rapidly throughout the western hemisphere, including the USA, Canada, Mexico, and the Caribbean and into parts of Central and South America. Before 1994, outbreaks of West Nile virus were sporadic and occurred primarily in the Mediterranean region, Africa, and east Europe. Since 1994, outbreaks have occurred with a higher incidence of severe human disease, particularly affecting the nervous system. In North America, the virus has caused meningitis, encephalitis, and poliomyelitis, resulting in significant morbidity and mortality. The goal of this Review is to highlight recent advances in our understanding of West Nile virus virology, ecology, clinical disease, diagnosis, and development of potential vaccines and antiviral therapies.

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Introduction

Many members of the genus *Flavivirus* within the family *Flaviviridae* cause substantial human disease, including West Nile, dengue, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus.[1] Neuroinvasiveness is a common feature of flavivirus infections where *Culex* mosquitoes are the predominant mosquito vector. Since 1999, about 19 525 cases of West Nile disease have been reported in the USA, of which 8 606 (44%) caused neuroinvasive disease with 771 fatalities (3·9% of all; 9·0 % of neuroinvasive disease; table).[2] The 2002–03 epidemics were the largest outbreaks of meningitis or encephalitis ever reported in the western hemisphere, making West Nile virus the dominant vector-borne viral pathogen in North America. Seroprevalence varied from 0·3% in Alberta, Canada, to 9·5% in Nebraska, with even higher rates in local areas following the large scale outbreak in 2003.[3,4] In North Dakota, USA, in 2003, blood donor screening suggested that 735 000 individuals were infected with West Nile virus, with 0·4% of these presenting with neuroinvasive disease.[5] Interestingly, almost no morbidity and mortality have been recognised in human beings, equines, and birds in Latin America and South America, although two equine cases were recently reported in Argentina.[6] The reasons for this are not clear but hypotheses include protection by antigenically cross-reactive flaviviruses,[7] decreased virulence of the circulating virus, high biodiversity in tropical regions leading to a dilution effect,[8] and decreased intensity of surveillance and diagnostic efforts.

West Nile virus ecology

West Nile virus is maintained worldwide in an enzootic cycle, transmitted primarily between avian hosts and mosquito vectors. Mosquitoes of the genus *Culex* are implicated as the predominant vectors in the enzootic cycle throughout the range of the virus' distribution.[9] The predominant mode of perpetuation of the virus in a temperate environment over adverse seasons is likely to be by vertically infected diapausing (ie, physiologically enforced dormancy between periods of activity) adult mosquitoes.[10–12] However, the transmission cycle may also be reinitiated through reintroduction of virus by migratory birds from their winter breeding grounds or from locations where the virus may be transmitted all year round,[13] or by recrudescence from low concentrations of virus in avian tissue.[14]

Non-vector routes of transmission have been described—eg, oral transmission in birds,[14] cats, and other vertebrates.[14–16] Although human beings are most commonly infected by mosquito bites, transmission can also occur through blood transfusion,[17] transplantation,[18] breast milk,[19] and intrauterine transmission.[20] Organ recipients are at very high risk for neuroinvasive disease after blood transfusion, donor transmission, or community exposure.[21] In 2002, transmission through organ transplantation was recognised for the first time in four patients who were recipients of organs from a common donor.[18] Risk of neuroinvasive disease in an organ recipient infected with West Nile virus is estimated as 40% (95% CI 16–80%)[22] compared with less than 1% in the general population. Administration of Omr-IgG-am, an intravenous immunoglobulin product with high-titre neutralising antibodies to West Nile virus, after disease onset did not ameliorate symptoms in patients infected by organ transplantation.[23]

Molecular epidemiology

Phylogenetic analyses of global West Nile virus strains have revealed two distinct lineages (I and II). Lineage I strains are commonly involved in human and equine outbreaks. Phylogenetic analyses of West Nile virus isolated from the USA indicates that the virus remains highly conserved genetically and was successfully introduced only once into North America. A single conserved aminoacid change in the envelope gene

	1999	2000	2001	2002	2003	2004	2005
Cases	62	21	66	4156	9862	2539	3000
Neuroinvasive disease (%)	95·2	90·5	97·0	70·9	29·1	45·0	43·1
Deaths	7	2	9	284	264	100	119

Data from the US Centers for Disease Control and Prevention as of Jan 10, 2006.[4]

Table: **Human illness from West Nile virus infections reported in the USA, 1999–2005**

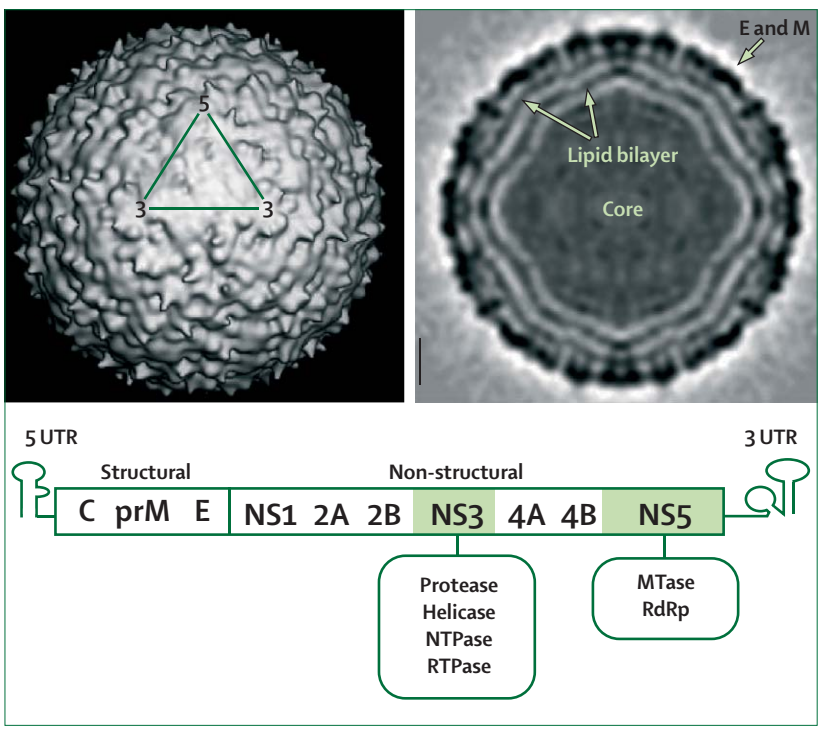

Figure 1: **West Nile virion and genome**
The virus structure as reconstructed by cryo-electron microscopy. One asymmetric unit of the icosahedron is indicated by the triangle on the surface shaded view. The central section of the reconstruction shows the concentric layers of mass density. Reproduced with permission from the American Association for the Advancement of Science.[27] NS3 is a serine protease (with NS2B as a cofactor), 5′-RNA triphosphatase (RTPase), nucleoside triphosphatase (NTPase), and RNA helicase. NS5 is an RNA-dependent RNA polymerase (RdRp) and N-7 and 2′O-methyltransferase (MTase) involved in the methylation of the 5′ RNA cap structure. E=envelope, m=membrane, UTR=untranslated region.

(V159A) is shared by most strains isolated since 2002 (North American dominant)[24,25] and is also found in old world strains of West Nile virus. This genotype first emerged in 2002 when the activity of West Nile virus increased expansively, and has effectively displaced the genotype originally introduced to the USA. Risk-assessment analyses suggest that human transport (mosquitoes on aeroplanes) may be an important pathway for introduction to new areas, including Hawaii[9] and the Galapagos Islands,[26] in addition to transport by birds.

Basic virology

Cryoelectron microscopy showed that virions of West Nile virus have icosahedral symmetry of 50 nm in diameter, with no surface projections or spikes (figure 1).[27] The outermost layer contains the viral envelope and membrane proteins embedded in a lipid bilayer, forming the envelope of the virion. Inside the envelope is the nucleocapsid core, which consists of multiple copies of the capsid protein and genomic RNA. The West Nile virus genome is a single-stranded RNA of plus-sense polarity (ie, mRNA). The viral genome is about 11 000 bp in length, consisting of a 5′ untranslated region (UTR), a single long open reading frame, and a 3′ untranslated region. The open reading frame encodes a polyprotein that is processed by viral and cellular proteases during and after translation into three structural proteins (C, premembrane or membrane, and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Structural proteins are mainly involved in viral particle formation, whereas non-structural proteins function in viral replication, virion assembly, and evasion of host innate immune response.

Host proteins and flavivirus resistance

Host proteins have important roles in West Nile virus replication. Translation elongation factor alpha (EF-1α) binds specifically to the 3′ stem-loop of the plus-sense genomic RNA,[28] wherease host proteins TIAR and TIA-1 bind to the 3′ stem-loop of the minus-sense RNA.[29] The functions of such host–protein binding to viral RNA are unclear. The Src family kinase c-Yes was recently reported to be important for maturation of West Nile virus particles.[30] Inbred mouse strains exhibit significant difference in their susceptibility to flavivirus infection. Resistance to virally induced morbidity and mortality in mice is flavivirus-specific and is inherited as a single dominant allele. 2′-5′-oligoadenylate synthetase 1b, *OAS*, was identified as a candidate for the flavivirus-resistance phenotype.[31] Compared with the resistant mice, susceptible mice produce an OAS1b protein lacking 30% of the C-terminal sequence, resulting in the inactivation of the OAS/RNase L pathway. Consequently, a large amount of virus is produced in the susceptible mice. Sequence comparison of *OAS* and *RNase L* genes between patients with West Nile disease and controls showed multiple single-nucleotide polymorphisms, but no insertion, deletion, or nonsense mutations.[32] More recently, Scherbick and colleagues found that, although *RNase L* has a role in the antiviral response to West Nile virus, the activation of *RNase L* is not a major component of the *OAS1b*-mediated flavivirus-resistance phenotype.[33]

Evasion of host innate immune response

Flavivirus non-structural proteins suppress host antiviral immune responses; however, the molecular details are unknown. Expression of the dengue virus-2 NS4B and, to a lesser extent, NS2A and NS4A proteins, results in down-regulation of interferon-β-stimulated gene expression.[34] The inhibition could be mediated by the 125 N-terminal aminoacid residues of dengue virus-2 NS4B protein.[35] During West Nile virus infection, the host response limits viral spread through the activation of the interferon regulatory factor 3 pathway.[36] Interferon-α signalling and STAT-2 translocation to the nucleus were inhibited when Kunjin (a naturally attenuated subtype of West Nile virus) NS2A, NS2B, NS3, NS4A, and NS4B, but not NS1 and NS5 proteins were individually expressed.[37] Furthermore, West Nile virus replication prevents the phosphorylation and activation of JAK1 and Tyk2.[38] Kunjin replicon accumulated an aminoacid substitution (Ala30Pro) in NS2A that could reduce the

NS2A-mediated inhibition of the interferon response.[39] Dengue and langat viral NS5 protein could also block the interferon-stimulated JAK–STAT pathway independently of STAT1 activation and translocation into the nucleus.[40]

Clinical presentation

Most individuals infected with West Nile virus are asymptomatic. Symptoms may develop in 20–40% of people with West Nile virus infection.[41,42] The incubation period is 2–14 days before symptom onset. Most patients that are symptomatic present with flu-like symptoms (West Nile fever). West Nile virus is characterised by fever, headache, malaise, myalgia, fatigue, skin rash, lymphadenopathy, vomiting, and diarrhoea.[43] Less than 1% of infected individuals develop severe neuroinvasive diseases, a majority of them can be primarily classified into three clinical syndromes: West Nile meningitis, West Nile encephalitis, and acute flaccid paralysis. Clinical features of these syndromes may overlap in the same patient. Whether these syndromes are different aspects of a continuous clinical spectrum or distinct entities is unknown. A recent study of 228 patients reported that most patients with neuroinvasive disorders can be classified as either having West Nile meningitis or West Nile encephalitis and patients with the latter have a higher mortality rate and more severe complications.[44] Thus, the distinction between these syndromes seems to be clinically useful. In addition, other syndromes have also been described, including rhabdomyolysis,[45,46] chorioretinitis,[47] myositis,[48] and autonomic nerve involvement.[49] Although uncommon, additional neurological syndromes may occur, such as hepatitis, pancreatitis, myocarditis, orchitis, uveitis, and vitritis.[43,50,51] Patients with West Nile virus may present with two, three, or even more of these syndromes at the same time. Here we focus on meningitis, encephalitis, and acute flaccid paralysis.

West Nile meningitis and encephalitis

West Nile meningitis usually presents with fever and signs of meningeal irritation, such as headache, stiff neck, nuchal rigidity, and photophobia.[50–53] Kernig's and Brudzinski's signs may be found on physical examination.[54] When the infectious process involves the brain parenchyma, West Nile encephalitis develops and additional clinical features can appear. Patients may have an altered level of consciousness, disorientation, and focal neurological signs and symptoms (eg, dysarthria, seizures, tremor, ataxia, involuntary movements, and parkinsonism).[50–53] These variable clinical presentations show the selectivity of neuroinvasion in West Nile virus in certain cell populations, such as substantia nigra in brainstem, basal ganglia,[53] and cerebellum.[49] All of these clinical features have also been reported in patients with other flavivirus infections; thus, these flaviviral infections are difficult to differentiate clinically in the acute phase.[53]

Most patients with West Nile fever completely recover within days to months.[50,55,56] However, patients with West Nile encephalitis have a poorer outcome than those with West Nile mengitis. In a recent study of 221 patients with West Nile virus infection, the 12 patients who died during hospitalisation had West Nile encephalitis. 25% of surviving patients with West Nile encephalitis returned home without the need for increased care, compared with 76% of patients with West Nile meningitis.[50] Patients with West Nile encephalitis with movement disorders had a favourable outcome in an earlier study.[53]

Acute flaccid paralysis

Clinical features in patients with West Nile meningitis or encephalitis are usually familiar to many physicians and prompt them to search for a viral cause. However, acute flaccid paralysis may not be familiar to some clinicians, particularly when it occurs in the absence of meningitis or encephalitic signs and symptoms,[57] resulting in difficulties for an accurate diagnosis.

About 10% of the hospitalised patients in the 1999 New York City outbreak had acute flaccid paralysis[57]. However, the underlying cause (poliomyelitis) for this acute paralysis was not recognised until 2002.[58–60] The selective lesion of spinal anterior horns by West Nile virus was documented decades ago.[61] In fact, by inoculating West Nile virus intramuscularly or intraperitoneally, encephalomyelitis was induced in patients with cancer. Autopsies from these patients with encephalomyelitis induced by West Nile virus clearly showed all typical pathological features that are now described in North American patients with neuroinvasive diseases from West Nile virus.[62,63] These studies were the first clinical and pathological documentations for West Nile virus poliomyelitis. Unfortunately, these studies have been largely ignored for many years because West Nile virus was not an epidemic in North America. During the summer of 2002 through to 2005, there a substantial number of cases of West Nile virus infection with acute flaccid paralysis.[57–59,64–66] The main clinical feature was acute asymmetric flaccid paralysis (dark limbs; figure 2).[57,66,67] Paralysis reached a plateau within hours in most patients. There was minimal or no sensory disturbance; most patients had substantial muscle ache in the lower back; bowel and bladder functions were disturbed in some patients.[58,68]

1–2 weeks before the onset of muscle weakness many patients have mild flu-like symptoms including headache, fever, malaise, gastrointestinal upset, skin rash, and some patients have neck rigidity and changes in mental status. However, half of our patients did not have viral prodrome or any meningitis or encephalitic signs.[57] Most patients were healthy before the illness and had no evidence of immunological suppression.[57,65] On neurological examination of patients with acute flaccid paralysis, most cranial nerves are usually normal. However, there may be facial weakness in more than half of these patients. Flaccid limb weakness is conspicuous.

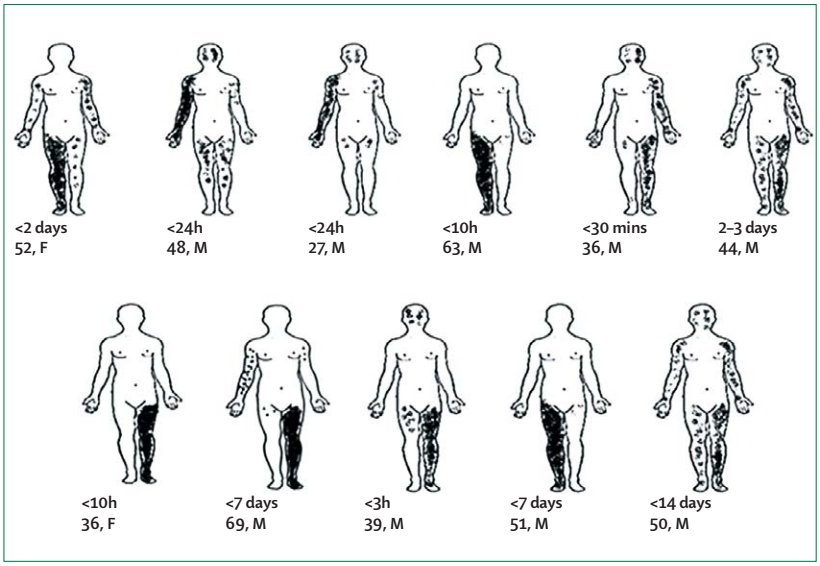

Figure 2: **Clinical features induced by West Nile virus paralysis**
The main clinical features of our patients are illustrated. Weak limbs at the peak of paralysis are darkened. Degree of darkness corresponds to the severity of weakness. Duration of weakness and characteristics (age [years] and sex [F=female, M=male]) are listed below each patient. Reproduced with permission from Elsevier.[70]

Muscular atrophy develops in the late phase of the illness. Sensory examination is normal or minimally affected. Deep tendon reflex can be diminished in severely paralysed limbs.[51,52,57,65]

Data on recovery from paralysis induced by West Nile virus are scant. Among patients hospitalised in New York and New Jersey in 2000, only a third regained the ability to walk within a year.[69] In another study, three patients with acute flaccid paralysis needed to use a wheelchair after an 8-month follow-up.[53] Although no detailed quantitative assessment of motor function was described in these studies, they suggest an unfavourable outcome. However, a study in 2002 showed that patients infected with West Nile virus had a remarkable variation in recovery.[66] Some patients recovered completely within weeks. A group of our patients with paralysis induced by West Nile virus were followed up for about 2 years using certain quantitative measures. Highly variable recoveries were reported and it was noted that the initial severity of the illness did not always predict a severe final outcome.[67,70] Prediction of whether patients with paralysis will develop a post-West Nile virus poliomyelitis syndrome similar to postpolio syndrome is not yet possible.

Although there have been patients that were diagnosed as having Guillain-Barre syndrome (GBS) or GBS-like disease, [54,65,71] all cases with detailed electrophysiological data do not support a demyelinating neuropathy.[51,57,59,66,72,73] There was only one exception in which detailed nerve conduction studies showed the development of a neuropathy with conduction velocities below 35m/s in one patient with West Nile virus infection.[71] Therefore, it is reasonable to state that demyelinating neuropathy has been extremely unusual among patients in the USA since 1999.

Clinical laboratory features

To diagnose West Nile virus infection, serum from patients should be tested for IgM antibodies against the virus (usually by ELISA), which are usually indicative of a recent West Nile virus infection. Blood samples that are collected between the eighth and 21st day after onset likely to give the best yield. IgM antibodies are only detectable 8 days post-symptom onset in some patients.[74,75] Thus, there may be a negative result from a blood sample obtained before the eighth day after symptom onset. After the 21st day, the titre of IgM could decline.

Anti-West Nile virus IgM can persist for 1 year or longer in some patients; a single positive test is not necessarily associated with the patient's current illness. In addition, cross-reactivity with other flaviviruses may occur. Thus, a definitive diagnosis requires testing two serum samples taken at least 2 weeks apart. A fourfold rise in antibody titre in acute and convalescent sera will confirm the diagnosis. A definitive diagnosis of West Nile meningitis, encephalitis, or poliomyelitis can also be made by looking for IgM antibodies in the cerebrospinal fluid from patients with pertinent neurological symptoms. Alternatively, neurological syndromes of those with West Nile virus can be examined by detection of West Nile virus RNA with reverse transcriptase PCR of cerebrospinal fluid, or by immunohistochemical demonstration of West Nile antigens in neural tissues; however, these alternative approaches are rarely used in clinical practice.[43,76]

Other abnormal findings in the cerebrospinal fluid include raised concentrations of lymphocytes and proteins. Neutrophils may contribute (at least 50%) in the early phase of the illness. West Nile meningitis and encephalitis have similar degrees of pleocytosis. However, patients with West Nile encephalitis tend to have higher concentrations of total protein in the cerebrospinal fluid and usually experience a more severe outcome.[77]

MRI is usually normal in most patients. However, abnormalities have also been described,[51,57] such as lesions in the focal cerebral hemispheric white matter (figure 3), pons,[51,53] substantial nigra,[53] and thalamus with increased signal intensity on T2-weighted images. An important MRI finding is the focal abnormal signal intensity within the anterior horns.[51,57] In this subgroup of patients, the level of abnormal spinal MRI findings corresponds to the paralysis. This observation provides strong evidence for the selective damage of the anterior horn of the spinal cord.[57] Abnormal MRI findings in the spinal roots have also been reported.[51] A similar finding of root enhancement was also reported in one of our patients (figure 3). Even though this could represent a primary spinal root involvement of West Nile virus (radiculopathy or radiculitis),[51] it is more likely that this MRI change is a

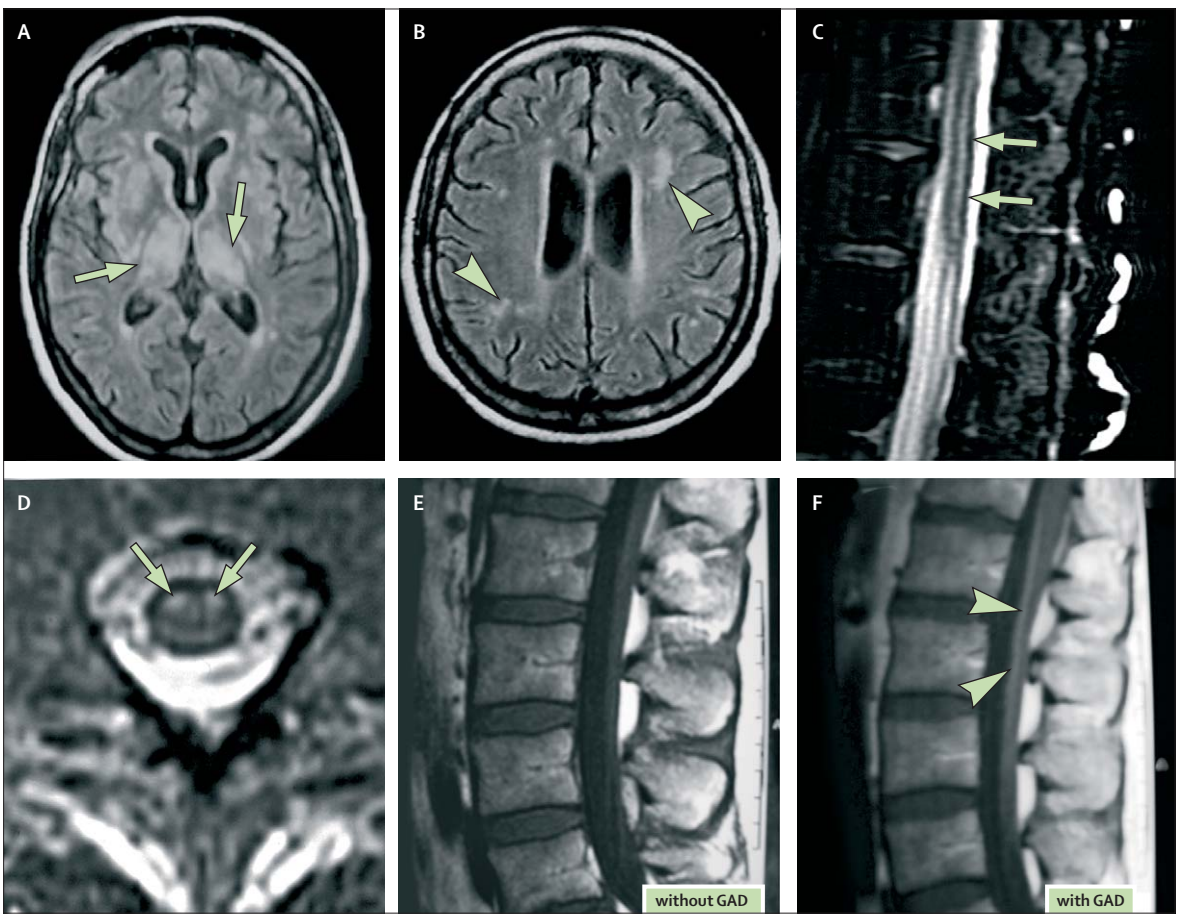

Figure 3: **Abnormal MRI findings in patients with West Nile virus**
Axial FLAIR (fluid-attenuated inversion recovery) image of the brain from a 57-year-old woman with encephalitis (A) shows abnormal signals in bilateral thalamus and other areas of basal ganglion. Focal white matter lesions are also seen (B). Sagittal T2-weighted MRI of the lumbar spinal cord (C); abnormal signal intensity (arrows) is conspicuous within the cord. A transverse view of the cord at the mid-lumbar level (D); abnormal signal intensity (arrows) is confined to the anterior horns; T1-weighted lumbar spine MRI from a patient with both meningitis and acute flaccid paralysis shows no discernable abnormality (E); however, after giving GAD contrast, spinal roots are significantly enhanced (F). Parts C and D reproduced with permission from Wiley.[57]

result of axonal degeneration secondary to spinal motor neuron loss. The second possibility is supported by our autopsy study that showed pathological changes consistent with an acute Wallerian degeneration in the spinal roots.[70]

Electrophysiological studies are helpful for the diagnosis of paralysis induced by West Nile virus.[51,57,66,72] Motor-nerve conduction studies may reveal severely reduced amplitudes of compound muscle action potentials in symptomatic limbs. However, if the nerve conduction study is done in the early phase of the illness, compound muscle action potentials can be normal because Wallerian degeneration can take 7–10 days to complete. Nerve conduction velocities are usually preserved, and sensory nerve conduction is typically normal. Needle electromyography shows severe denervation in muscles of weak limbs and its corresponding paraspinal muscles. Taken together, these abnormalities in the paralysed limbs localise the lesions to the anterior horn motor neurons or their ventral nerve roots. The localisation is typically consistent with the aforementioned MRI findings.

Neuropathology and pathogenesis of clinical syndromes

West Nile virus is thought to initially replicate in dendritic cells after being bitten by an infected mosquito. The infection then spreads to regional lymph nodes and into the bloodstream.[2] The way in which the virus invades the nervous system is still unknown; retrograde transport along peripheral nerve axons has been proposed.[70,78] On the basis of animal models, a Toll-like receptor-dependent inflammatory response could be involved in brain penetration of the virus and neuronal injury.[79] CNS expression of the chemokine receptor CCR5 and its ligand CCL5 are prominently upregulated by the West Nile virus, associated with CNS infiltration of CD4+, CD8+ T cells, NK1.1+ cells, and macrophages that express CCR5.[80] In mice, in response to the West Nile virus infection, neurons

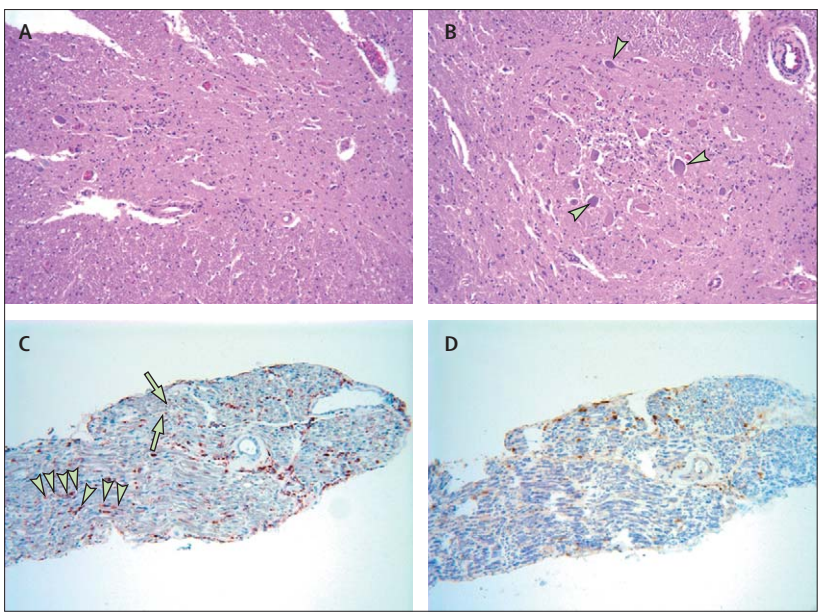

Figure 4: **Pathology of the spinal cord and roots from an autopsy of a patient infected with West Nile virus**
Haematoxylin and eosin-stained sections of the thoracic spinal cord from an autopsy (magnification 250X). Motor neurons at the anterior horn are almost depleted (A). By contrast, most motor neurons (arrowheads) in another segment of the thoracic spinal cord are well preserved (B). A few motor neurons seem swollen (arrows in B). In addition, conspicuous inflammatory cells infiltrate diffusely in both segments of the cord. Immunohistochemistry of the spinal root with antibodies against CD68 (brown-colour dots in C) and CD3 (brown-colour dots in D; magnification 250X). Abundant inflammatory cells and axonal ovoids are present in the root (arrowheads and arrows). CD68 cells (brown-color in C) are numerous and distributed in linear arrays along the degenerating axons (the array of arrowheads). These features are highly suggestive of a secondary inflammatory reaction to an ongoing Wallerian degeneration in the root. Reproduced with permission from Elsevier.[70]

secrete chemokine CXCL10, which recruits effector T cells via the chemokine receptor CXCR3.[81] A recently identified receptor for West Nile virus, αVβ3 integrin, provides new insights into how the virus invades different tissues.[82]

Histological CNS findings of West Nile virus infection are usually characterised by perivascular lymphoplasmacytic infiltration, microglial nodules, reactive proliferation of astrocytes, variable necrosis, and neuronal loss with predilection to structures like the thalamus, brainstem, and cerebellar Purkinje cells.[48,61–64,74] These variable anatomical involvements explain different clinical presentations.

The selective destruction of anterior horn motor neurons seems particularly relevant to understanding the pathogenesis of neuroinvasiveness. This selective lesion with inflammatory infiltration has been confirmed in several autopsy studies (figure 4)[64,70] and West Nile virus antigens were localised in the anterior horn.[83] This localisation is consistent with early pathological studies[84,85] as well as the findings from people intramuscularly inoculated with West Nile virus.[62] Moreover, almost all flaviviruses cause motor neuron damage at the anterior horns; however, poliomyelitis is much less common in patients with other non-West Nile flaviviruses.[85–89] On the basis of clinical presentation, neuroimaging, and pathological findings, motor neuron damage at the anterior horn of the spinal cord is probably the major contributor to West Nile virus paralysis.

Recovery among patients that have been paralysed is remarkably variable. The reason for this variation is still unclear. Data suggest that the variation may be caused by different degrees of motor neuron or motor unit loss.[70]

Diagnosis

The diagnosis of West Nile virus paralysis should be considered whenever clinical presentation of viral meningitis or encephalitic and acute asymmetric paralysis with normal sensory examination occurs during the seasons when mosquito-borne diseases might occur. Absence of viral prodrome does not exclude the diagnosis.[57,65] Electrophysiological studies and neuroimaging can be helpful. A definitive diagnosis for neuroinvasive diseases usually requires a positive IgM antibody test from the serum or cerebrospinal fluid in an appropriate clinical setting. Confirmation of recent infection is confounded by long-lasting IgM antibodies; IgM can last up to 16 months.[90] Thus, caution should be taken when interpreting these laboratory results.

All flaviviruses of the Japanese and tick-borne encephalitis complex can cause meningitis and encephalitis. Because meningitis and encephalitis with these viruses are clinically similar, at least in the acute phase, differential diagnosis has to rely on serological assays on sera or cerebrospinal fluid or on PCR testing.

In patients with acute flaccid paralysis, differential diagnosis can include GBS, myopathy, neuromuscular junction disorders, and other virus-related motor neuron diseases. Sensory disturbance and slow conduction velocities from nerve conduction studies may be sufficient to differentiate those with GBS and West Nile virus paralysis. Acute motor axonal neuropathy, as a subtype of GBS, can closely imitate West Nile virus paralysis, but is less problematic in the USA since acute motor axonal neuropathy is extremely rare in this country and it symmetrically affects limbs.

In addition, all these flaviviruses affect the lower motor neurons. Flaccid weakness and needle electromyography abnormalities consistent with damage of the lower motor neurons have been reported in St Louis encephalitis,[88,90] Japanese encephalitis,[87] Murray Valley encephalitis,[91] and Powassan encephalitis.[92] Pathological studies have shown inflammatory involvement in the anterior horns in Japanese encephalitis, nearly identical to that seen in poliovirus myelitis.[83,86] Russian Spring-Summer encephalitis causes similar pathological abnormalities;[93] however, its clinical presentation is unique and is characterised by cervical cord motor neuron involvement and conspicuous head-drop due to neck extensor denervation. These infectious agents should be considered when dealing with a patient with an acute lower-motor-neuron disorder. Because it is challenging to clinically

differentiate among these diseases, epidemiological data may be critical for the diagnosis, and specific serological or PCR tests are commonly necessary.

Vaccine development

Human vaccines for flavivirus infections are currently available only for yellow fever, Japanese encephalitis, and tick born encephalitis.[1] Because the premembrane and envelope proteins are highly antigenic and elicit strong and long-lasting immune responses, multiple approaches have been explored to deliver these antigens into animals for vaccine development. The first approach is based on chimeric viruses delivering West Nile virus antigens. The yellow fever 17D vaccine strain, which has been safely used for large-scale human immunisation for over 60 years, is an excellent vector for delivering protective antigens of flaviviruses. Chimeric vaccines were constructed by replacing the yellow fever 17D premembrane and envelope genes with the corresponding genes of other flaviviruses, including the virus of Japanese encephalitis,[94] dengue,[95] and West Nile.[96] Similarly, chimeric viruses of West Nile virus combined with dengue virus 2 or 4 are immunogenic, and provide complete protection against lethal West Nile virus challenges.[97,98] In mice, expression of West Nile virus premembrane and envelope genes, through attenuated measles virus, conferred protection from a lethal dose of West Nile virus.[99] Among these vaccine candidates, chimeric viruses of West Nile virus and yellow fever virus and with dengue virus are in phase I clinical trials. The recent results of clinical trials showed that yellow fever chimeric virus elicits strong immune responses without apparent adverse events after a single dose.[100] After inoculation of 30 healthy adults with 10^5 plaque-forming units or 15 people with 10^3 plaque-forming units (n = 15) of West Nile–yellow fever chimeric virus, all adults developed neutralising antibodies to West Nile virus, and most developed a specific T-cell response.

The second approach is based on immunisation of animals with recombinant viral proteins, inactivated West Nile virus, or DNA that expresses viral antigens. Formalin-inactivated West Nile virus, a recombinant canarypox virus vector, and a DNA plasmid expressing West Nile virus premembrane and envelope proteins have been developed and approved for equine use.[101–103] These results agreed with previous findings that expression of tick-borne or Japanese encephalitis premembrane and envelope proteins in mammalian cells produced empty virus particles that contained proteins embedded in the lipid bilayer without a nucleocapsid. Mice immunised with such West Nile virus particles were protected from infection.[104] Furthermore, direct immunisation with recombinant West Nile virus envelope protein alone protected mice and horses against subsequent infections.[105] In a hamster model, animals immunised with 1 μg of recombinant envelope protein were completely protected from lethal challenge with West Nile virus for at least 6 months. No viraemia or clinical disease were reported, and high titres of viral neutralising antibodies were elicited.[106] These promising results warrant further assessment of recombinant envelope as a vaccine candidate in monkeys and human beings.

The third approach is based on attenuated West Nile virus isolates. Kunjin virus is a naturally attenuated West Nile virus subtype that could provide protective immunity in mice against the virulent New York West Nile virus strain.[107] An attenuated non-epidemic West Nile virus strain (lineage II) was also shown to be an effective vaccine against virulent epidemic strain (lineage I) in mice.[108] For other flaviviruses, introduction of deletions into the capsid protein of TBEV has been reported as a promising way to create attenuated, yet highly immunogenic, flaviviruses.[109] Deletions within the 3′ untranslated region were also shown to attenuate dengue virus[110] and tick-borne encephalitis virus.[111] These approaches could alternatively be used for development of West Nile virus vaccines.

Antiviral therapy

There is no specific regime currently available for treatment of flavivirus infections. Although several compounds have been reported to inhibit recombinant West Nile virus enzymes or to suppress virus in cell culture, few of them have shown in vivo efficacy. Antibody-based therapy has yielded the most promising results. Passive administration of monoclonal antibodies has previously shown prevention and alleviation of St Louis encephalitis,[112] Japanese encephalitis,[113] and encephalitis caused by yellow fever virus.[114] Intravenous immunoglobulin that contains high titers of West Nile virus antibodies seemed effective in patients with West Nile encephalitis in an open-label study; however, these findings require further confirmation in controlled studies.[115] Humanised monoclonal antibodies against the West Nile virus envelope protein have shown efficacy in mice, even when given as a single dose at day 5 post-infection and when the virus has already infected the CNS.[116] The efficacy of these monoclonal antibodies against the envelope protein still needs to be determined in monkeys and human beings.

Two antisense phosphorodiamidate-morpholino-oligomers (PMOs) were reported to potently inhibit West Nile virus infection in cell culture.[117] A new Arg-rich peptide was conjugated to the PMO for efficient cellular delivery. Because one PMO targets a flavivirus-conserved RNA sequence that is essential for viral RNA synthesis, this PMO had a broad spectrum of anti-flavivirus activities in cell culture.[117] These PMOs could partially protect mice from West Nile disease when given at day 5 post-infection (Shi PY, unpublished). These results warrant further improvement in anti-sense PMO-based therapies.

Interferon α-2b is currently under clinical trial for treatment of patients with West Nile virus-mediated

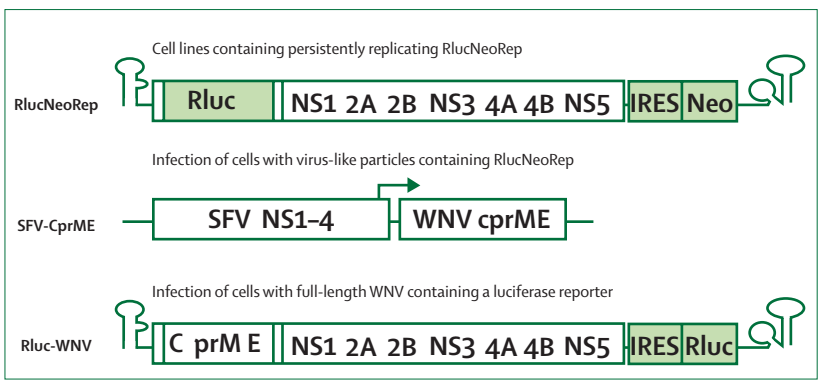

Figure 5: **Cell-based, high-throughput assays for West Nile virus drug discovery**
For each assay, a *Renilla* luciferase (Rluc) gene was engineered into a replicon (containing a deletion of three structural genes) or into a full-length viral genome to monitor viral replication. Potential inhibitors could be identified through suppression of luciferase signals upon compound incubation. A reporting cell-line assay (top): the cell line has a persistently replicating replicon that contains dual reporter genes, luciferase and neomycin phosphotransferase, resulting in RlucNeoRep; the assay allows screening of inhibitors of all targets involved in viral replication. Virus-like particle infection assay (middle): Semliki forest virus vector containing viral non-structural proteins (SFV NS1–4) was used to express West Nile virus (WNV) structural proteins C-prM-E under the control of the SFV 26S subgenomic promoter (SFV-CprME). Transfection of RlucNeoRep-containing cells with SFV-CprME packages RlucNeoRep RNA into VLPs. Infections of naive cells with such particles results in replication of RlucNeoRep, yielding Rluc signals; this assay allows screening of inhibitors of viral entry and viral replication. A full-length reporting WNV infection assay (bottom): a luciferase gene driven by an EMCV IRES was engineered at the 3′-untranslated region of the genome, resulting in Rluc-WNV. Infection of cells with Rluc-WNV generates Rluc signals; this assay allows screening of inhibitors of all steps of viral infection cycle, including entry, replication, and assembly.

meningoencephalitis. Interferon α-2b has inhibited in vivo West Nile virus replication.[118] Mice with a defective receptor for interferon α or interferon β (and therefore defective in interferon response) showed higher mortality, shorter survival time, and altered cellular tropism of infection in comparison with wild-type mice inoculated with the West Nile virus.[119] Treatment of primary neurons infected with West Nile virus with interferon β increased neuronal survival. There was substantial recovery in the neurological function of five patients with West Nile virus-CNS disease treated with interferon α-2b soon after symptom onset.[120] However, one patient treated with interferon α-2b died, probably because of delayed diagnosis and treatment, as well as other complications.[121] More controlled studies are needed to demonstrate the efficacy of the interferon treatment.

Three cell-based high-throughput assays have been developed for the discovery of a drug for West Nile virus (figure 5).[122] A luciferase reporter was engineered into a subgenomic replicon (containing a deletion of viral structural genes) and into a full-length West Nile virus. Replication of a reporting replicon or virus in cells results in expression of luciferase, which can be used to monitor antiviral activities of potential inhibitors. Alternatively, viral structural proteins could package the luciferase-expressing replicon into virus-like particles. Infection of cells with such reporting virus-like particles could also be used to screen potential inhibitors. Screening compound libraries with these high-throughput assays has identified a new triaryl pyrazoline compound that could inhibit flaviviruses in cell culture, including West Nile, dengue, and yellow fever virus.[123] Mode-of-action analyses in West Nile virus showed that the compound specifically suppressed viral RNA synthesis. The in vivo efficacy and the specific target of the triaryl pyrazoline inhibitor are unknown.

Conclusions

Improvement in our understanding of flavivirus virology, ecology, and pathogenesis will substantially contribute to the prevention and treatment of flavivirus infections. For example, crystallographic studies have shown that envelope proteins undergo a sequential structural change during the fusion-activating transition.[124] Small-molecule inhibitors could be developed to block the structural switch that is essential for viral–host membrane fusion. The findings that flavivirus proteins antagonise host innate immune response have provided opportunities to develop novel antiviral therapy and vaccines for flaviviruses. For antiviral therapy, inhibitors that block the activity of interferon antagonism of viral proteins would allow the innate antiviral response to effectively suppress viral infection and, therefore, reduce viral burden. For vaccine development, mutant flaviviruses containing aminoacid changes in viral proteins to suppress its function on inhibiting interferon response could be generated through manipulation of infectious cDNA clones. The mutant viruses are competent in replication, but defective in interferon antagonism. Inoculation of animals with such mutant viruses is expected to elicit a strong immune response due to viral replication. However, since the viruses are sensitive to the host's interferon inhibition, they will be eliminated by the host without causing disease.

A rapid and virus-type-specific serological assay is needed for clinical diagnosis of flavivirus infection. One potential means to improve the current flavivirus diagnosis is to use luciferase-reporting flaviviruses for the standard antibody-mediated neutralising assay. Similar to the luciferase-expressing West Nile virus (figure 5), other flaviviruses containing the luciferase reporter could be generated. Neutralisation of flaviviruses will be indicated by luciferase signals within 24 h post-infection. The reporting virus-based assay will avoid the time-consuming plaque assay (which requires about a week depending on the type of flavivirus) and should substantially shorten the time to diagnosis. In support of this idea, reporting replicon-containing viruses have recently been reported for measuring antibody-mediated neutralisation of West Nile virus.[125]

It is important to understand the ecology of West Nile virus, including the causes of spatial and temporal variation in transmission, the roles of host reservoir competence, acquired immunity in West Nile virus amplification, and the impact of climate on vector ecology and viral replication. Finally, despite extensive studies on the phenotypic presentation of West Nile virus neuroinvasive diseases, the pathogenic mechanism of these diseases is still unclear. Unravelling the mechanism

Search strategy and selection criteria

References for this Review were identified by searches of PubMed using the terms "West Nile virus" and "flavivirus" from 1950 to March 2006. Additional references and book chapters that were cited in relevant papers were also used. Abstracts from conferences were also included. Only references in English were used. References were selected on the bases of originality and relevance.

of selective invasion of West Nile virus into spinal motor neurons may provide a therapeutic target for blocking of West Nile disease. A combination of basic science and translational research will greatly facilitate the prevention and treatment of West Nile virus.

Contributors

All authors contributed equally to the preparation of this Review. LDK wrote sections on the ecology and epidemiology of West Nile virus. JL wrote sections on clinical presentation and diagnosis. PYS wrote sections on virology, evasion of innate immune response, development of vaccines and antiviral therapy.

Conflicts of interest

We have no conflicts of interest.

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Potential of three aquatic predators to control mosquitoes in the presence of alternative prey: a comparative experimental assessment

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Abstract. Predator-induced control of pests depends on the predator's preference for the target pest over naturally co-occurring prey species. We compared the larvivorous efficiency of three common freshwater predators: mosquitofish (*Gambusia affinis*; Baird and Girard, 1854), dragonfly naiads (*Zyxomma petiolatum*; Rambur, 1842) and copepods (*Mesocyclops aspericornis*; Daday, 1906) on different instars and the relative abundances of the mosquito *Anopheles stephensi* (Liston, 1901) in the presence of alternative cladoceran prey, either *Moina macrocopa* (Straus, 1820) or *Daphnia similoides* (Hudec, 1991). Larval removal rate decreased with increasing larval size and instar stage. The maximum consumption rate was by mosquitofish, followed by dragonfly naiads and copepods. The presence of either of the alternative prey significantly reduced larval consumption by all three predators, except in the *D. similoides*–mosquito larvae combination for naiads. Mosquitofish and copepods preferred early instars of the mosquito. Prey selectivity indices for early mosquito instars against *D. similoides* did not differ between mosquitofish and copepods, whereas naiads had significantly lower index values than the other two predators. Considering the negative impacts of mosquitofish on native assemblages, that is, its invasiveness and its lower selectivity for mosquito larvae, our results suggest that the feasibility of using copepods in large-scale control programs needs to be evaluated.

Additional keywords: biocontrol, foraging, preference, prey selection, vector control.

Introduction

Mosquitoes are important pests that affect human health and well-being throughout the world. Mosquito-transmitted diseases, such as malaria, dengue and yellow fever, continue to be among the most serious vector-borne diseases in tropical and subtropical regions. An extension of the geographical boundary of mosquito-borne diseases has been recorded in many parts of the world, including Australia and Asia (Hati 1997; Sharma and Hamzakoya 2001). In particular, *Culex annulirostris* (Skuse, 1889) is a known vector of flaviviruses, such as Murray Valley encephalitis virus and Kunjin virus, and alphaviruses, such as Ross River virus and Barmah Forest virus (Weinstein *et al.* 1995; Russell and Dwyer 2000).

Vectors of malaria in Asia are *Anopheles stephensi* and *Anopheles culicifacies* (Giles, 1901). *Anopheles stephensi* is a subtropical species and an important vector of human malaria throughout the Middle East and South Asia region (Al-Tikrity 1964). The control of *A. stephensi* has become a major task owing to the development of resistance against all commonly used insecticides, including the new generation of insecticides such as synthetic pyrethroids (Sharma and Hamzakoya 2001). Until now, the main strategy of control of *A. stephensi* has been indoor spraying of residual insecticides. Despite the persistent use of synthetic insecticides, mosquito populations have not been reduced significantly and still retain their potential to support the pandemic. Chemical control measures have proven hazardous to humans and ecosystems (Milam *et al.* 2000; Kumar and Hwang 2006). In view of the current restrictions on the use of pesticides and continuing problems with chemical resistance, biological control is an important option for mosquito control. Environmentally sensitive methods of mosquito control are required to manage these pests and the threat of arboviral and malarial diseases (Russell 1999; Russell and Dwyer 2000; Kumar and Hwang 2006). Biological control techniques are relatively inexpensive and appropriate for developing countries, where human resources are more available than funds for chemical control alternatives.

Although a variety of biological control agents ranging from viruses to amphibians have been tried for mosquito control and have been shown to be successful in the laboratory, under natural conditions there is still no consistently successful and acceptable mosquito population reduction achieved by biocontrol agents (Lounibos and Frank 1994; Kumar and Hwang 2006). The use of biological control agents based on a national commitment to reduce pesticide reliance is under intense scrutiny because of suspicion that agents introduced for control purposes might exert

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negative impacts on biodiversity and contribute to the decline of non-target species.

The use of natural predators to control immature individuals of vectors with aquatic larval stages has been explored (Lounibos *et al.* 1997; Bellows 2001; Willems *et al.* 2005). Natural predators of mosquito larvae include the tadpole stages of amphibians (Mokany 2007), planktivorous fishes, dragonfly naiads, hemipteran water bugs such as *Notonecta* and *Anisops* (Blaustein 1998; Hampton 2004), dytiscid beetles such as *Rhantus*, *Ilybius* and *Agabus* spp. (Lundkvist *et al.* 2003; Aditya and Saha 2006), malacostracans, anostracans, cyclopoid copepods and triclad flatworms (Kumar and Hwang 2006). At present, only fishes (particularly Poeciliidae) are widely used as biocontrol agents, but their success in controlling vector populations remains highly variable (Blaustein 1992). Many predators that have been shown to be highly successful in eliminating target prey in the laboratory do not show a similar response in their natural habitats. Deviations from laboratory findings are attributed to the permanence of water (Lester and Pike 2003) and the predator's preference for the target prey in the presence of an alternative prey community co-occurring in the natural habitat (Arthington and Lloyd 1989).

Mosquito-breeding habitats include pools, paddy fields, shallow wetlands, rain-filled puddles, floodplains and other waterlogged areas that experience recurrent dry phases of varying duration (Williams 1987, 1997). In general, the hydroperiod in habitats corresponds to the breeding periods of mosquitoes, and these habitats become ideal breeding sites for mosquitoes, particularly for the genera *Aedes* and *Anopheles* (Laird 1988). James (1961, 1966) reported that immature mosquitoes are less exposed to natural enemies in temporary ponds compared with permanent ponds. Considering the availability of alternative prey along with the heterogeneity of the habitats in temporary pools, temporary aquatic habitats are depicted as so-called 'enemy-free' spaces (Brendonck *et al.* 2002). In many mosquito-breeding habitats, the following predators of mosquito larvae have been noted: cyclopoid copepods (Marten *et al.* 1994*a*, 1994*b*; Zoppi de Roa *et al.* 2002), dytiscid beetles such as *Rhantus signatus signatus* (Fabricius, 1775) (Fischer *et al.* 2000; Campos *et al.* 2004), *Rhantus sikkimensis* (Regimbart, 1899) (Aditya *et al.* 2006*a*, 2006*b*), the notonectid bugs *Notonecta maculata* (Fabricius, 1794) and *Anisops sardea* (Kirkaldy, 1904) (Murdoch *et al.* 1984; Eitam *et al.* 2002; Kiflawi *et al.* 2003; Eitam and Blaustein 2004), the mosquitofish (Wurtsbaugh *et al.* 1980; Howe *et al.* 1997), and the cannibalistic mosquito larvae *Toxorhynchites* (Theobald 1901) (Collins and Blackwell 2000). These predators regulate mosquito populations and even influence their oviposition and habitat-selection behaviour (Kiflawi *et al.* 2003; Blaustein *et al.* 2004).

The appropriate selection of effective natural enemies has become increasingly important for the success of biological control programs (Denoth *et al.* 2002; Müller and Brodeur 2002; Hoelmer and Kirk 2005). Therefore, the crucial decision in a biological control program is the selection of agents that are not recognised by gravid females, and that can establish themselves in the temporary habitats and eliminate immature individuals of the proposed target pest immediately after hatching. For the operational use of biocontrol agents, it is important that they can be easily introduced to mosquito-breeding sites, that they disperse and build up viable populations (Caltagirone 1981; Bellows 2001; Headrick and Goeden 2001), and that they do not pose any threat to humans (Rishikesh *et al.* 1988). The predator's preference for the proposed target pest from a wide spectra of natural prey species and habitat specificity are important criteria for selection as a potential biocontrol agent. In this respect, the cyclopoid copepod *Mesocyclops thermocyclopoides* (Harada, 1931) (Kumar 2003; Kumar and Rao 2003) and the dytiscid beetles *Colymbetes*, *Ilybius*, *Rhantus* and *Agabus* spp. (Lundkvist *et al.* 2003) have been shown to prefer mosquito larvae over other arthropod prey. In addition, the predator's adaptability to the introduced environment (Carlson *et al.* 2004) and its overall interaction with native organisms need to be considered before introduction (Denoth *et al.* 2002).

Although the basic ecological concepts (predator–prey interactions) underlying biological control have been elucidated, their relevance and applicability to mosquito control have not been investigated in a scientific way (Rao and Kumar 2002; Kumar and Rao 2003; Speczíar 2004). The suitability of a predator for mosquito control depends to a great extent on its effectiveness as a predator, and this effectiveness should be evaluated under laboratory conditions before field trials (Lloyd *et al.* 1986) by addressing the following questions: (1) how do different natural predators differ in their preference for mosquito larvae; and (2) to what extent is their removal efficiency of mosquito larvae influenced by the availability and relative abundance of alternative prey? To answer these questions, the present study compared the biocontrol potential of three larvivorous predators that commonly co-occur in the wetlands of tropical and subtropical regions, the mosquitofish *Gambusia affinis* (Baird and Girard, 1854), the cyclopoid copepod *Mesocyclops aspericornis* (Daday, 1906) and naiads of the dragonfly *Zyxomma petiolatum* (Rambur, 1842), against the larvae of the urban malaria-vector *A. stephensi* (Liston, 1901) in the presence of alternative cladoceran prey, either *Moina macrocopa* (Straus, 1820) or *Daphnia similoides* (Hudec, 1991).

Materials and methods

Collection, culture and preparation of experimental animals

All experimental animals used in the present study were cultured in the laboratory. The cladocerans *Moina macrocopa* and *Daphnia similoides* were isolated from plankton samples collected from outdoor pools by filtering the pool water through a plankton net of 200-μm mesh. Cultures were maintained in autoclaved tap water and fed the green alga *Chlorella vulgaris* (Beijerinck, 1890) at densities of $2–2.5 \times 10^6$ mL^{-1}. These cladoceran species commonly co-occur in high densities (Kumar and Hwang 2008) in the natural habitats of copepods and constitute a food source for predatory copepods.

Eggs of the mosquito species *Anopheles stephensi* were obtained from the National Institute of Communicable Diseases, Delhi, India, and larval cultures were maintained separately in a room with constant optimal conditions (temperature of 25 ± 2°C, relative humidity of 70 ± 5% and a 14 h light : 10 h dark photoperiod). Mosquito eggs were placed in enamel trays within sealed containers in de-chlorinated tap water to which

Table 1. Body size, dry mass, taxonomic group and microhabitat of the prey and predator species used in the experiments
Body size measurements are means ± s.e. ($n = 15$–20)

Ecological group	Species	Taxonomic group	Mean length (mm)	Dry mass (μg)	Microdistribution
Predator	Mosquitofish	Cyprinodontiformes: Poeciliidae	47.23 ± 6.5	–	Throughout water column (higher density in surface waters)
	Naiads	Odonata: Libellulidae	17.32 ± 1.64	–	On the bottom/clinging to the sides
	Copepod	Copepoda: Cyclopoida	1.13 ± 0.13	–	Throughout water column
Prey	*Anopheles stephensi* (instar-I; early)	Diptera: Culicidae	0.79 ± 0.03	3.48	Water surface
	A. stephensi (instar-III: middle)	Culicidae	1.38 ± 0.03	46.50	Water surface
	A. stephensi (instar-IV: late)	Culicidae	1.86 ± 0.05	185.20	Water surface
	Daphnia similoides	Branchiopoda: Cladocera	1.44 ± 0.05	18.52	Throughout water column
	Moina macrocopa	Cladocera	0.81 ± 44.42	4.66	Throughout water column

the standard diet consisting of yeast extract and finely powdered dog biscuits (2 : 3) was added for emerging larvae and their subsequent stages. Freshly emerged pupae were transferred from larval containers to bowls containing water and placed into cages covered with mosquito netting where they were allowed to hatch into adults. The emerging adults were fed daily with sucrose solution soaked in cotton balls and also weekly with rodent blood to promote egg development. A tray containing water-soaked filters was placed inside the cage as a substrate for ovipositing females to lay eggs within these cages. Eggs were removed daily and placed into fresh, sterilised, covered larval containers until the larvae emerged. All containers and water used for propagation were autoclaved before washing and reuse.

Ovigerous females of *Mesocyclops aspericornis* were collected with a plankton net from outdoor concrete tanks, and placed individually in borosilicate glass Petri dishes (90 mm diameter) containing 40 mL of autoclaved tapwater and kept at 25 ± 2°C and a photoperiod of 14 h light : 10 h dark until the eggs developed. Newly hatched nauplii were reared separately on an algal diet of *Chlorogonium elongatum* (Dangeard 1899). All cultures and animals used in the experiments were developed from a single female. Stock cultures were maintained in 1000-mL beakers and kept in a BOD incubator at a temperature of 25 ± 1.5°C under diffuse fluorescent illumination. The cultures in the beakers were changed daily, with fresh *C. vulgaris* as food and a mixture of mosquito larvae and cladocerans. Nine outdoor water-filled concrete tanks were initially fertilised with mustard oil cakes and inoculated with the alga *C. vulgaris*. These tanks supported higher densities of cladocerans and cyclopoid copepods. Dragon fly naiads were collected from these outdoor cement tanks using a dip net. Prior to the experiment, naiads were kept in 10 cm × 7 cm glass dishes, each containing 500 mL of autoclaved tap water, for 5–6 days and reared on a mixed diet of cladocerans and mosquito larvae.

To culture mosquitofish, egg-carrying females were collected with fish nets from an ornamental pond at the University of Delhi. Over 5 days, 10–15 offspring from each of 20 broods were used in the experiment. Approximately 50 fish (~six broods) were raised individually in 500-mL glass dishes and fed a mixture of cladoceran and mosquito larvae. For all cultures and experiments, the room temperature was maintained at 25 ± 2°C. The experimental animals and their characteristics are listed in Table 1. Each animal was used only once in an experiment. The predators used in the experiments were exposed to all test prey species before testing because they were cultured using combinations of the same prey species as food.

Experimental designs and protocols

All experiments, with six replicates for each treatment, were conducted in 10 cm × 7 cm glass dishes, each containing 500 mL of autoclaved tap water with a fixed number of prey (Table 2). To measure larval consumption rates, predators were offered early, middle and late instars of mosquito larvae, and for the prey-choice experiment, either early (I–II) or late (IV) instar larvae were used in combination with either of the two cladocerans at three relative proportions (0.25, 0.5 and 0.75). A series of short-duration preliminary experiments were conducted to investigate the feeding efficiency (number of larvae ingested per time unit) of mosquitofish, naiads and copepods on different instars of the mosquito larvae. On the basis of these experiments, the initial densities for each prey type and the test duration were chosen so that a substantial number of prey remained unconsumed at the end of the experiment. The starvation duration was chosen based on the gut clearance rate of each predator species. Only non-ovigerous prey were used to exclude any possible increase in prey density as a result of reproduction during the test period. The beakers were placed in an unilluminated incubator at a temperature of 25 ± 1.5°C, and the predators were allowed to feed for a fixed period of time (Table 2). At the end of the experiment, the prey and predator individuals remaining in each beaker were preserved in buffered formalin and subsequently counted. Random sampling of a number of beakers did not reveal any prey losses during the test period as a result of death by natural causes. However, the guts of the predators were dissected to analyse the gut contents to confirm the estimates of consumption.

Table 2. Experimental protocols used for mosquito larval consumption and prey-selection experiments by the three predators

Predator species	Test no.	Prey combination and numbers					Starvation duration (h)	Test duration (h)
		Mosquito larvae			Alternative cladoceran prey			
		Early instars (I–II)	Middle instars (III)	Late instars (IV)	*Daphnia similoides*	*Moina macrocopa*		
I. Larval consumption rates								
Mosquitofish	1	200	–	–	–	–	3	1
		100	–	–	100	–	3	1
		100	–	–	–	100	3	1
	2	–	200	–	–	–	3	1
		–	100	–	100	–	3	1
		–	100	–	–	100	3	1
	3	–	–	200	–	–	3	1
		–	–	100	100	–	3	1
		–	–	100	–	100	3	1
Naiads	4	200	–	–	–	–	12	6
		100	–	–	100	–	12	6
		100	–	–	–	100	12	6
	5	–	200	–	–	–	12	6
		–	100	–	100	–	12	6
		–	100	–	–	100	12	6
	6	–	–	200	–	–	12	6
		–	–	100	100	–	12	6
		–	–	100	–	100	12	6
Copepods	7	200	–	–	–	–	12	12
		100	–	–	100	–	12	12
		100	–	–	–	100	12	12
	8	–	200	–	–	–	12	12
		–	100	–	100	–	12	12
		–	100	–	–	100	12	12
	9	–	–	200	–	–	12	12
		–	–	100	100	–	12	12
		–	–	100	–	100	12	12
II. Prey-choice experiment								
Mosquitofish	10	50	–	–	150	–	3	1
		100			100		3	1
		150			50		3	1
	11	50	–	–	–	150	3	1
		100				100	3	1
		150				50	3	1
	12	–	–	50	150		3	1
				100	100		3	1
				150	50		3	1
	13	–	–	50		150	3	1
				100		100	3	1
				150		50	3	1
Naiads	14	50	–	–	150	–	12	6
		100			100		12	6
		150			50		12	6
	15	50	–	–	–	150	12	6
		100				100	12	6
		150				50	12	6
	16	–	–	50	150	–	12	6
				100	100		12	6
				150	50		12	6
	17	–	–	50	–	150	12	6
				100		100	12	6
				150		50	12	6

(Continued)

Table 2. (Continued)

Predator species	Test no.	Prey combination and numbers					Starvation duration (h)	Test duration (h)
		Mosquito larvae			Alternative cladoceran prey			
		Early instars (I–II)	Middle instars (III)	Late instars (IV)	*Daphnia similoides*	*Moina macrocopa*		
Copepods	18	50	–	–	150	–	12	12
		100			100		12	12
		150			50		12	12
	19	50	–	–	–	150	12	12
		100				100	12	12
		150				50	12	12
	20	–	–	50	150	–	12	12
				100	100		12	12
				150	50		12	12
	21	–	–	50	–	150	12	12
				100		100	12	12
				150		50	12	12

Larval consumption rate

The purpose of this experiment was to elucidate the consumption rates of the three predators of mosquito larvae as a function of instar stage and the presence of alternative prey. The consumption of mosquito larval instars I–II (early; 0.79 ± 0.025 mm), instar III (middle; 1.38 ± 0.029 mm) and instar IV (late; 1.86 ± 0.045 mm) by mosquitofish, naiads and copepods was recorded with mosquito larvae alone and in the presence of cladocerans, either *D. similoides* or *M. macrocopa* (i.e. three predator × three larval instars × three treatments (alone, in the presence of *D. similoides* or in the presence of *M. macrocopa*) × six replicates (Table 2)).

Prey-choice experiment

A series of prey-choice experiments was conducted to investigate the selectivity of the three predators for early and late instar larvae as a function of alternative prey species and their relative abundance in the medium (Table 2). Early (I–II)/late instars (IV) *v.* cladocera choice tests (early instars of *M. macrocopa*; early instars of *D. similoides*) were conducted for all three predators in three consecutive phases. A combined density of 200 prey individuals per litre was used in three prey proportions (0.25, 0.5 and 0.75). Prey were individually counted and placed at set concentrations in each dish containing 500 mL of water. These prey densities are commonly recorded in lakes and ponds in Delhi (Kak 1998). The experimental protocol for each predator included two larval instars (early and late) × two alternative prey types (*D. similoides* and *M. macrocopa*) × three proportions × six replicates.

Data analyses

The relationships between the larval consumption rates of each of the three predators and changes in instar stages and larval size were examined by regression analyses. As the reduction of larval consumption rate with increasing body size of mosquito larvae did not follow a normal distribution, we used a power regression model equation (Box and Cox 1964) to represent our data. The larval consumption data were also subjected to a general linear model factorial univariate ANOVA, following Gill (2001), to assess the interaction of the variables (predator type × larval instars × alternative prey) as well as the effects of the individual factors on larval consumption rates. To identify homogeneous subsets of means, the ANOVA was followed by pairwise multiple comparisons using Tukey's honestly significant difference (HSD) test.

Prey selectivity was calculated using Manly's selectivity index (α_i) (Manly 1974), modified for the situation in which the predator consumes a substantial portion of the prey available and, hence, prey numbers in the medium decline with time, as in our experiments. The modified formula (Chesson 1983) is

$$\alpha_i = \frac{\ln(n_{i0} - r_i)/n_{i0}}{\sum_{j=1}^{m} \ln((n_{i0} - r_i)/n_{i0})}, i = 1, 2, 3, \ldots, m$$

where α_i is Manly's α (preference index) for prey type I; n_{i0} is the number of items of type i present at the beginning of a foraging bout. r_i is the number of items of food type i in the consumer's diet and m is the number of prey types.

Manly's α_i values range from 0 to 1. The α_i value for non-selective feeding is 0.5 with two prey type choice tests. Index values above 0.5 indicate positive selection (preference) and values below 0.5 indicate negative selection (avoidance). Deviations in selectivity index values from 0.5, the value for non-selective feeding, were tested for statistical significance using Hotelling's T^2 test (Hotelling 1931), a generalisation of the univariate t-test for multivariate data.

Using a general linear model (Gill 2001) and a factorial multivariate ANOVA (MANOVA) test (Gotelli and Ellison 2004), we tested the null hypotheses that the presence of alternative prey

and different relative prey proportions do not affect a predator's preference for mosquito larvae. The MANOVA identifies the independent variables (e.g. predators, presence of alternative prey and relative abundance of prey) that best differentiate preference for mosquito larvae (Gill 2001). Among-group (H) and within-group (E) sum of squares and cross-product (SSCP) matrices were created and four test statistics (Pillai's trace, Wilk's lambda, Hotelling–Lawly's trace and Roy's greatest root) were generated from the H and E matrices (Scheiner 2001). As Pillai's trace, Wilk's lambda and Hotelling–Lawly's trace converge to a similar *P*-value, despite having differences in their degrees of freedom (Olson 1976; Scheiner 2001), we used Hotelling–Lawly's trace and Roy's greatest root tests for further interpretation. To evaluate which values of a factor contributed most to the explanation of the selectivity for mosquito larvae, post-hoc univariate pair-wise multiple comparisons using Tukey's HSD tests were carried out. To test prey switching mechanisms at all prey combinations, the observed and expected number of mosquito larvae consumed were subjected to regression analyses. The null hypothesis is that the ratio of two alternative prey consumed ($P_1 : P_2$) in a given time period is constant and independent of the relative availabilities of the two prey. We applied the formula for prey switching (Landry 1981; Kiørboe *et al.* 1996) in which the proportionality constant (C) (Landry 1981) was calculated using the formula:

$$\frac{P_1}{P_2} = C(N_1/N_2)$$

where N_1 and N_2 represent the densities of two prey species and P_1 and P_2 are the numbers of the two prey species consumed in a given time period. Increasing values of C (proportionality constant) with increasing values of N_1/N_2 imply prey switching (Landry 1981; Kiørboe *et al.* 1996).

Results

Larval consumption rate

All three predators used in this experiment were able to capture early, middle and late mosquito instars. The rates of removal of mosquito larvae decreased with increasing larval size and instar stage (Fig. 1) in all three predators. Although the observed responses of the predators to increasing larval size suggest a negative exponential, the second-order negative power equations gave a better fit (higher r^2 value) (Fig. 1). Mean larval consumption rates were significantly higher on early and middle instars than on late larval instars (Table 3) for all three predators (Fig. 2). In terms of the biomass as well as the numbers, the average consumption rate was highest in the mosquitofish, followed by the dragonfly naiads and the copepods. The average consumption rates of early larval instars when present as exclusive food were 23.8 ± 1.3 (83.3 μg), 9.5 ± 0.5 (33.3 μg) and 2.6 ± 0.2 (9 μg) larvae h^{-1} (mean ± s.e.) for the mosquitofish, dragonfly naiads and copepods respectively (Fig. 2).

The presence of alternative prey in the medium had a significant impact on the rate of predator-driven removal (Fig. 3) of mosquito larvae. The interactions among predator species, prey species and larval instar stages were also significant (Table 3). Different levels of reduction in larval consumption rates were achieved by *Daphnia similoides* and *Moina macrocopa* (Fig. 3), which was a function of the predator species and the instar stage. The mosquitofish and the naiads responded significantly to the presence of alternative prey (Fig. 2*a*, *b*; Table 4), and the response to *M. macrocopa* was higher than the response to *D. similoides* in terms of reduction in larval consumption (Fig. 3). For mosquitofish, either of the cladoceran species as an alternative prey significantly reduced the consumption of early or middle instar larvae (Fig. 2; Table 4). The reduction was significantly higher (Fig. 3*a*; Table 4) in the presence of *M. macrocopa* (20–40%) as an alternative prey. For the dragonfly naiads, the presence of *D. similoides* as an alternative prey did not elicit any significant effect ($P = 0.47$) on larval consumption rates, regardless of instar stages. However, the presence of *M. macrocopa* resulted in a significant reduction (60–70%, Fig. 3*b*; Table 3) in the larval consumption of early and middle instars. For the copepods, the presence of *D. similoides* elicited significantly higher removal rates (Table 4) of early and middle instars of *Anopheles stephensi* by the copepods (Fig. 3*c*). The presence of

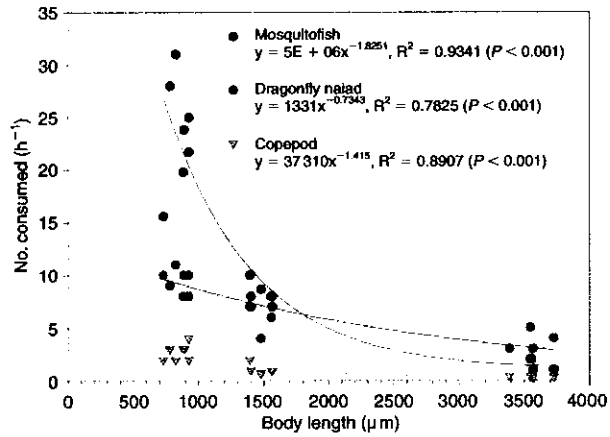

Fig. 1. Larval consumption rates (number) in mosquitofish, naiads and copepods as a function of the larval size of *Anopheles stephensi*. The trend lines were drawn using negative power equations.

Table 3. Results of the 3-way factorial ANOVA on the consumption rate of the larval instars I, III and IV of *Anopheles stephensi* alone, in the presence of *Daphnia similoides* and in the presence of *Moina macrocopa* by the three predators: mosquitofish, dragonfly naiads and copepods

Source	d.f.	*F*-value	*P*-value
Predator	2	510.301	<0.001
Instar stage	2	563.006	<0.001
Alternative prey	2	133.276	<0.001
Predator × instar	4	261.572	<0.001
Predator × alternative prey	4	29.114	<0.001
Instar stage × alternative prey	4	13.306	<0.001
Predator × instar × alternative prey	8	3.973	<0.001
Within groups (residual)	135		
Total	162		

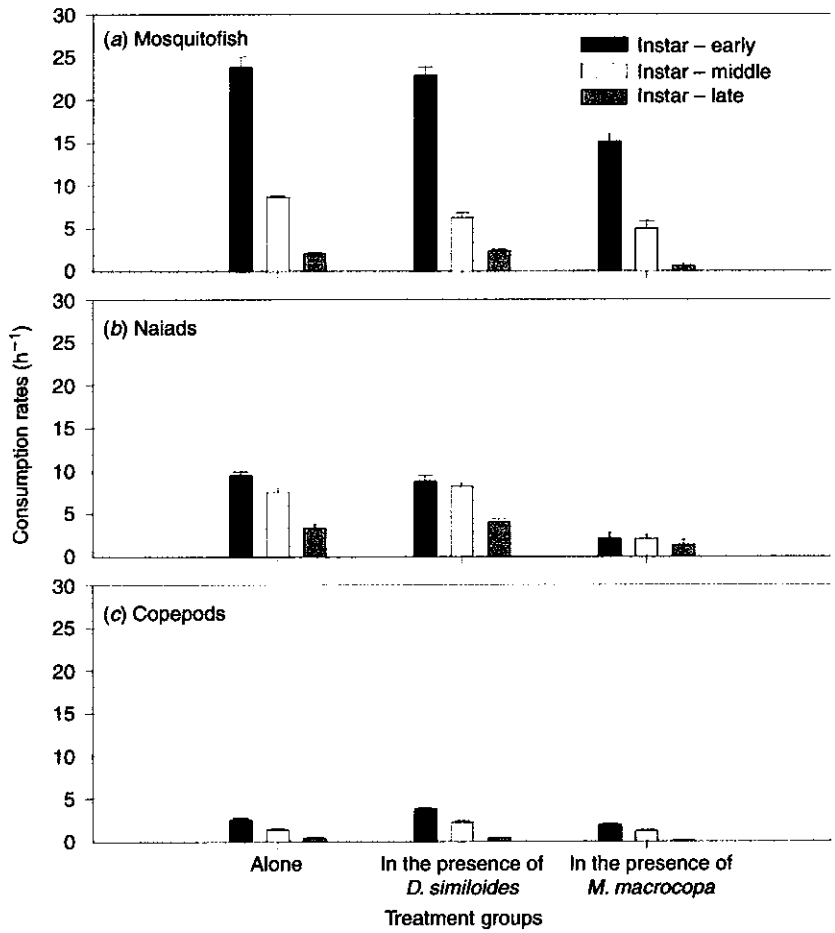

Fig. 2. Daily per capita prey consumption rates (mean ± s.e.) of early, middle and late instar larvae of *Anopheles stephensi* by the three predators: (*a*) mosquitofish, (*b*) dragonfly naiads and (*c*) the copepods in relation to larval instars of *A. stephensi* alone or in the presence of alternative cladoceran prey, either *Daphnia similoides* or *Moina macrocopa*.

M. macrocopa as an alternative prey did not induce any significant impact ($P = 0.11$) on larval removal by the copepods.

Prey-choice experiment

The three predators did not differ significantly in their selectivity for any of the larval instars except the early ones, *D. similoides* choice test (Roy's largest root, MANOVA, $P = 0.014$). However, differences in selectivity indices as a result of prey type and their relative abundances were highly significant (Hotelling–Lawly's trace test, $P < 0.001$; Tukey's HSD test, $P < 0.05$). Interactions of the predator with either prey type or the proportion of prey or both (predator × proportion; predator × type; and predator × type × proportion) were highly significant (MANOVA tests, $P < 0.001$; Table 5). The interactions between prey type × proportion were also significant in all cases (Table 5). These results indicate that the preference for mosquito larvae against co-occurring species differed among predators and the preference is a function of prey type, relative prey abundance, and their interactions.

Prey choice in mosquitofish

From a combination of early instar larvae and *D. similoides*, the mosquitofish positively selected larval instars and avoided the cladoceran *D. similoides*, regardless of their relative proportion in the medium (Fig. 4*a*; $\alpha_i = 0.89$–0.92, $P < 0.001$, MANOVA). However, in the early instars–*M. macrocopa* choice test, the differences in the selectivity indices were highly significant for prey type, proportion and their interactions (Fig. 4*b*; $P < 0.001$, MANOVA). The mosquitofish selected positively ($\alpha_i = 0.77 \pm 0.03$, $P < 0.001$, MANOVA) for *M. macrocopa* if offered this prey at a higher proportion, and randomly captured *M. macrocopa* ($\alpha_i = 0.601 \pm 0.06$; Hotelling's T^2 test) when given them at equal proportion in the early instars–*M. macrocopa* prey-choice test (Fig. 4*b*). The mosquitofish preferred early larval instars ($\alpha_i = 0.77 \pm 0.028$, $P < 0.05$, Hotelling's T^2 test) when the relative abundance of mosquito larvae was higher (0.75) than that of *M. macrocopa* (0.25).

Offered a combination of late instar (IV) larvae and *D. similoides*, the mosquitofish randomly captured ($\alpha_i = 0.62 \pm 0.08$;

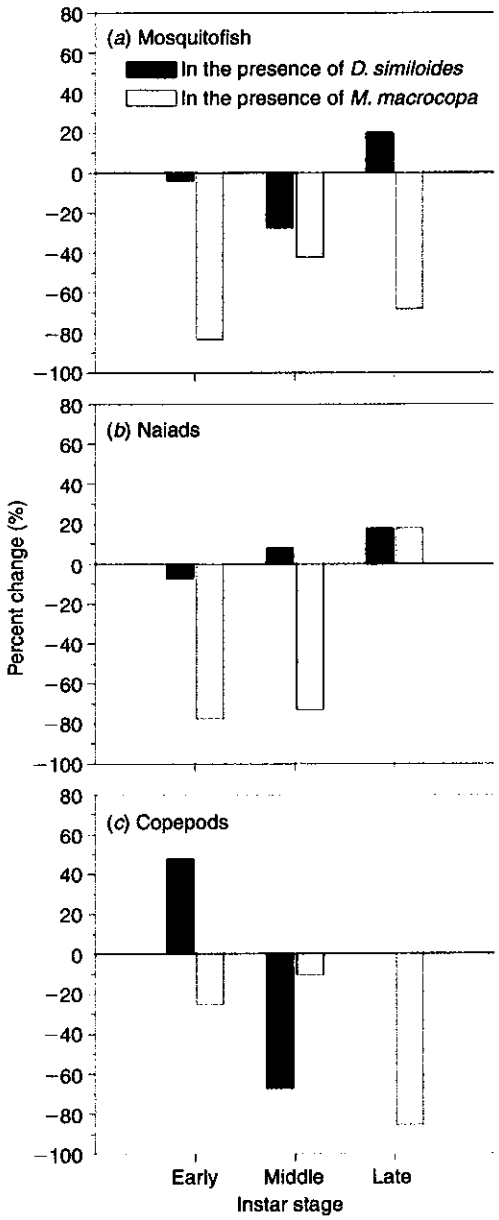

Fig. 3. Alternative cladoceran (either *Daphnia similoides* or *Moina macrocopa*) prey-induced percentage change in the consumption of early, middle and late instar larvae of *Anopheles stephensi* by the three predators: mosquitofish, naiads and copepods.

Fig. 4*c*) the late larval instars when the relative abundance of the larvae was lower (0.25), but positively selected at equal ($\alpha_i = 0.8 \pm 0.013$, $P < 0.01$, Hotelling's T^2 test) or at higher proportions ($\alpha_i = 0.7 \pm 0.02$, $P < 0.05$, Hotelling's T^2 test). Prey type, proportion and their interaction were significant in the late instars–*D. similoides* prey-choice test. In the late instars–*M. macrocopa* prey-choice test, late larval instars were avoided ($\alpha_i = 0.25$–0.373, $P < 0.05$, Hotelling's T^2 test; Fig. 4*d*) either at lower or at equal proportion, but randomly selected ($\alpha_i = 0.54 \pm 0.04$, Hotelling's T^2 test) when offered at a higher proportion.

Prey choice in dragonfly naiads

Mean differences in selectivity index values as a result of prey type, prey proportion and their interactions were significant in the dragonfly naiads ($P < 0.001$, MANOVA tests followed by Tukey's HSD tests). When offered a combination of early instar larvae and the larger cladoceran *D. similoides*, the naiads showed significant avoidance of the early larval instars at lower and at higher proportions ($\alpha_i = 0.18 \pm 0.046$ and 0.29 ± 0.02, respectively, $P < 0.001$, Hotelling's T^2 test; Fig. 5*a*). However, early instars were preferred when present at equal proportions with *D. similoides* ($\alpha_i = 0.68 \pm 0.04$, $P < 0.05$, Hotelling's T^2 test). When *M. macrocopa* was offered as an alternative prey, early larval instars were actively avoided ($\alpha_i = 0.07 \pm 0.024$ to 0.23 ± 0.056, $P < 0.01$, MANOVA; Fig. 5*b*), regardless of their relative proportion in the medium.

Offered a combination of late instars and either *D. similoides* or *M. macrocopa* as alternative prey, dragonfly naiads avoided late mosquito instars either at lower or at higher proportions ($\alpha_i = 0.28$–0.177, $P < 0.01$, MANOVA; Fig. 5*c*), but randomly selected prey when both prey types were offered in equal proportions ($\alpha_i = 0.395$–0.48, $P < 0.05$, Hotelling's T^2 test). In the *M. macrocopa*–late instars choice test, the late mosquito larval instars were actively avoided ($\alpha_i = 0.29 \pm 0.02$ to 0.07 ± 0.02, $P < 0.001$, Hotelling's T^2 test) and *M. macrocopa* was selected for ($\alpha_i = 0.71 \pm 0.02$ to 0.93 ± 0.02, $P < 0.001$, MANOVA; Fig. 5*d*), regardless of its relative proportion in the medium.

Prey choice in copepods

The mean differences in Manly's selectivity index values for copepods were significant for prey type ($P < 0.001$, MANOVA), prey proportion ($P < 0.05$, Hotelling–Lawly's Trace MANOVA test), and their interactions ($P < 0.001$, MANOVA tests). Early instars of mosquito larvae were positively selected ($\alpha_i = 0.87$–0.93, $P < 0.001$, MANOVA) against *D. similoides*, regardless of their relative abundance in the early instars–*D. similoides* choice test (Fig. 6*a*). Offered a combination of early instar larvae and *M. macrocopa*, the copepods preferred early instars to *M. macrocopa* at equal or at lower proportions of early larval instars ($\alpha_i = 0.81 \pm 0.03$, $P < 0.001$, MANOVA tests) (Fig. 6*b*). However, the mosquito larvae were neither selected for nor avoided when provided at lower proportion ($\alpha_i = 0.54 \pm 0.22$, $P = 0.096$, Hotelling's T^2 test).

In the late instar–cladocera choice tests, neither prey type nor the interaction of prey type with proportion was significant ($P > 0.6$, MANOVA tests). Therefore, the copepods neither avoided nor preferred late larval instar IV (Fig. 6*c*, *d*), regardless of the prey proportion in the late instars–cladocera combination.

Preference for mosquito larvae in three predators: a comparative evaluation

The three predators did not differ significantly in their selectivity for either of the larval instars except in the early instars–*D. similoides* prey-choice test (Roy's largest root, MANOVA,

Table 4. Pairwise multiple comparisons and differences between mean larval consumption rates by the mosquitofish, naiads and copepods when the larvae were given as food alone or in the presence of either *Daphnia similoides* or *Moina macrocopa* (using Tukey's HSD test; *$P < 0.05$)

Presence of alternative prey		Mean differences in larval consumption rates (per 3 h)			
		Predators combined	Mosquitofish	Naiad	Copepod
Alone	Presence of *D. similoides*	0.3148	2.39*	−0.44	−1.00*
	Presence of *M. macrocopa*	3.5185*	5.16*	5.0*	0.389
Presence of *D. similoides*	Alone	−0.3148	−2.39*	0.44	1.00*
	Presence of *M. macrocopa*	3.2037*	2.78*	5.44*	1.38*
Presence of *M. macrocopa*	Alone	−3.5185*	−5.16*	−5.00*	−0.39
	Presence of *D. similoides*	−3.2037*	−2.78*	−5.44*	−1.39*

Table 5. Results of a multivariate ANOVA (Hotelling–Lawly's trace and Roy's greatest root tests) for selectivity indices in the three predators (copepods, dragonfly naiads and mosquitofish) at three different prey proportions

Effect	Test statistics	Value	*F*-value	Numerator d.f.	Denominator d.f.	*P*-value
Intercept	Hotelling's trace	83.073	1806.833	4	87	<0.001
	Roy's largest root	83.073	1806.833	4	87	<0.001
Predator species	Hotelling's trace	0.163	1.751	8	172	0.090
	Roy's largest root	0.150	3.302	4	88	0.014
Prey type	Hotelling's trace	5.271	114.652	4	87	<0.001
	Roy's largest root	5.271	114.652	4	87	<0.001
Proportion	Hotelling's trace	2.244	24.122	8	172	<0.001
	Roy's largest root	2.172	47.792	4	88	<0.001
Predator type × prey type	Hotelling's trace	10.572	113.648	8	172	<0.001
	Roy's largest root	10.207	224.551	4	88	<0.001
Predator × proportion	Hotelling's trace	1.147	6.128	16	342	<0.001
	Roy's largest root	0.992	22.322	4	90	<0.001
Prey type × proportion	Hotelling's trace	0.236	2.535	8	172	0.012
	Roy's largest root	0.196	4.313	4	88	0.003
Predator type × prey type × proportion	Hotelling's trace	0.965	5.158	16	342	<0.001
	Roy's largest root	0.861	19.374	4	90	<0.001

$P < 0.05$). The significant interactions of predator with prey type, proportion or both ($P < 0.001$, MANOVA tests) indicate that the impacts of alternative prey species and their relative abundance in the environment are predator dependent. Mosquitofish and copepods did not differ ($\alpha_i = 0.732$; Fig. 7*a*) in their preference for early larval instars against *D. similoides*, whereas the mean selectivity indices (α_i) recorded for naiads were significantly lower ($P < 0.05$).

In the early instars–*M. macrocopa* preference tests, the copepods preferred ($\alpha_i = 0.768$, $P < 0.001$, MANOVA) early larval instars at lower and equal proportions of larvae and randomly utilised them at higher larval proportions, whereas the mosquitofish preferred early larval instars only at higher proportions (Fig. 7*b*). The naiads actively avoided the early instars of mosquito larvae, regardless of their relative abundance in the environment ($\alpha_i = 0.19 \pm 0.017$ to 0.22 ± 0.03, $P < 0.01$, MANOVA followed by Tukey's HSD test; Fig. 7*a*, *b*). Likewise, the naiads also actively avoided the mosquito larvae, regardless of alternative prey types and proportions in the late instar–cladocera choice tests. Mosquitofish avoided late larval instars when given at lower or equal proportions and only randomly captured mosquito larvae when offered at higher proportions in the late instars–*M. macrocopa* choice tests. When offered *D. similoides* as an alternative prey, the mosquitofish showed a higher selectivity index ($P < 0.05$; Fig. 7*c*) for late instars than either copepods or naiads. However, the copepods randomly killed late instars rather than either of the cladoceran prey (Fig. 7*c*).

Prey switching

Out of the 12 prey-choice experiments, the observed proportion of prey in the diet showed a significant positive correlation with the expected proportions in only two tests of the mosquito larvae–*M. macrocopa* choice tests for the mosquitofish (Fig. 8*a*). However, prey switching was significant for the early instars–*M. macrocopa* choice test only, in which the proportionality constant (*C*-values) increased significantly ($r^2 = 0.98$; $P < 0.001$) with increasing values of prey proportions (N_1/N_2) in the environment (Fig. 8*b*). This demonstrates that the ratio of mosquito larvae in the guts increased significantly with increasing relative availability of larvae. Therefore, our null hypothesis was rejected

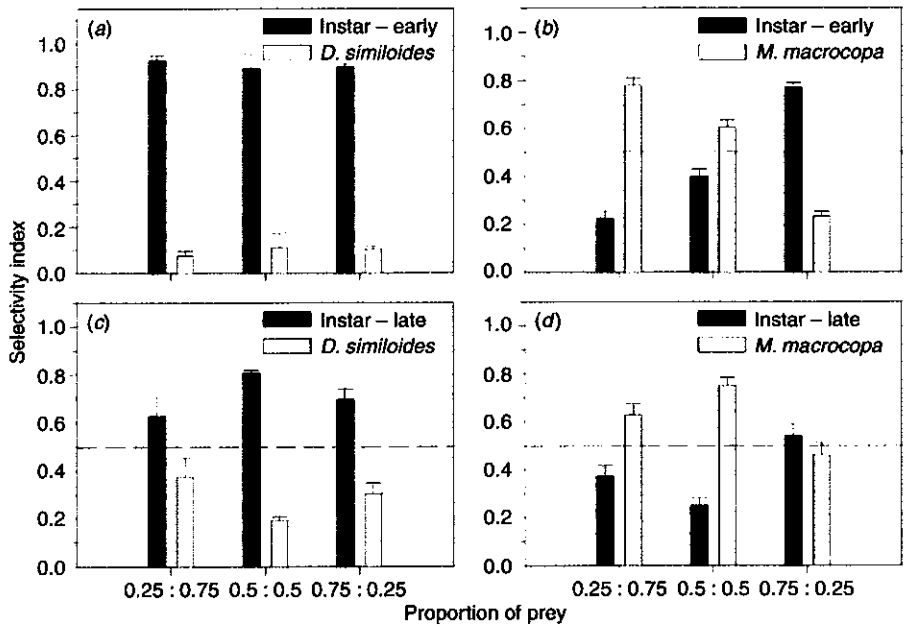

Fig. 4. Manly's selectivity index (α_i) value for the early (I–II) and late (IV) larval instars of *Anopheles stephensi* for mosquitofish offered a choice of (*a*) early instars *v. Daphnia similoides*, (*b*) early instars *v. Moina macrocopa*, (*c*) late instars *v. Daphnia similoides* and (*d*) late instars *v. Moina macrocopa* in three different proportions. Values shown are mean ± s.e. ($n = 6$). The dashed line indicates the value for non-selective feeding.

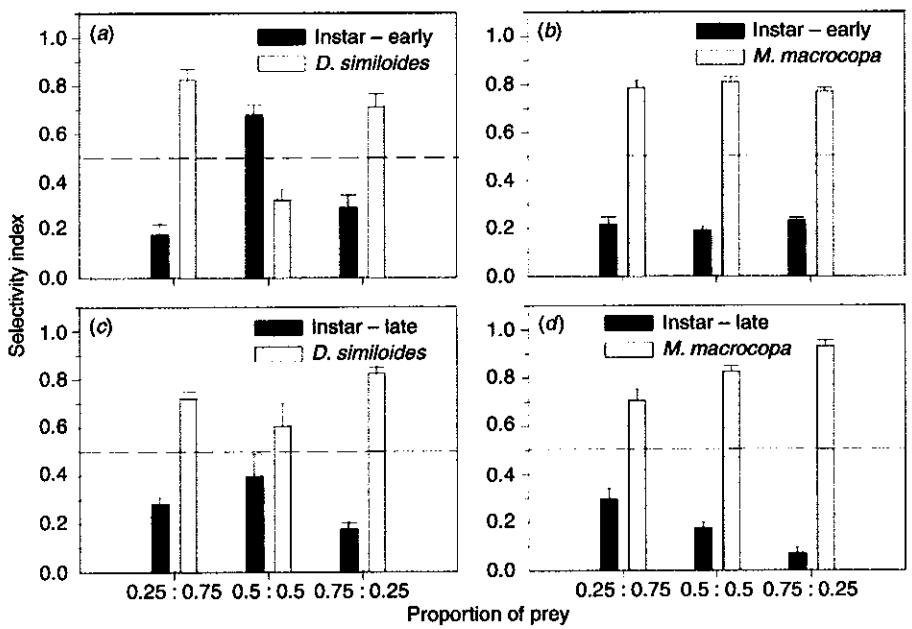

Fig. 5. Manly's selectivity index (α_i) value for the early (I–II) and late (IV) larval instars of *Anopheles stephensi* for dragonfly naiads offered a choice of (*a*) early instars *v. Daphnia similoides*, (*b*) early instars *v. Moina macrocopa*, (*c*) late instars *v. Daphnia similoides* and (*d*) late instars *v. Moina macrocopa* in three different proportions. Values shown are mean ± s.e. ($n = 6$). The dashed line indicates the value for non-selective feeding.

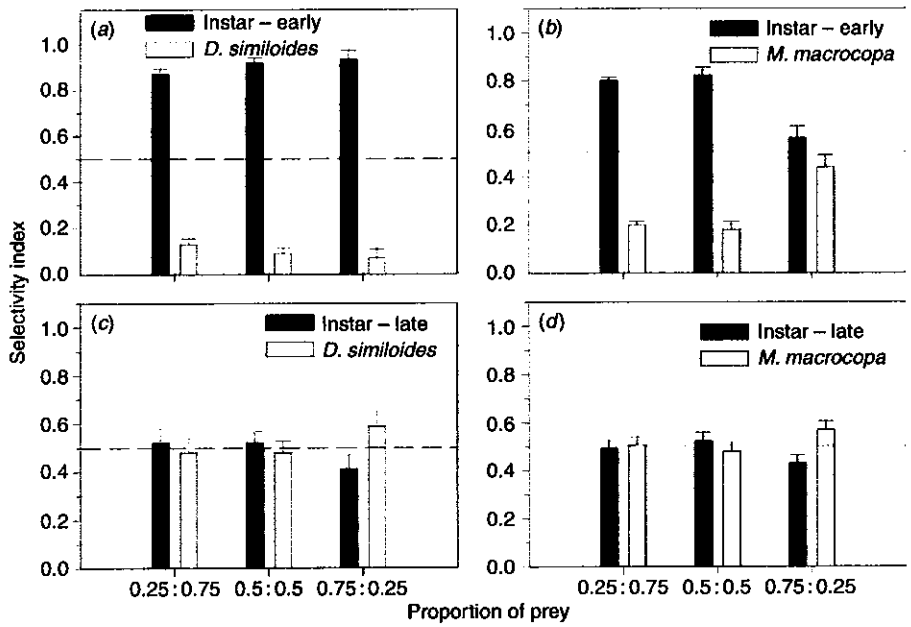

Fig. 6. Manly's selectivity index (α_i) value for the early (I–II) and late (IV) larval instars of *Anopheles stephensi* for the copepod *Mesocyclops aspericornis* offered a choice of (*a*) early instars *v. Daphnia similoides*, (*b*) early instars *v. Moina macrocopa*, (*c*) late instars *v. Daphnia similoides* and (*d*) late instars *v. Moina macrocopa* in three different proportions. Values shown are mean ± s.e. ($n = 6$). The dashed line indicates the value for non-selective feeding.

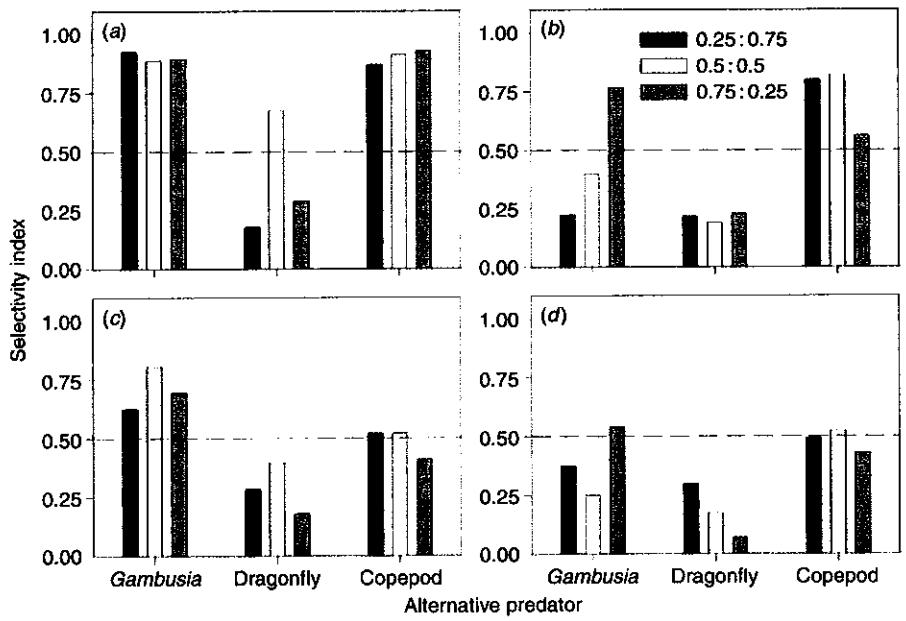

Fig. 7. Selectivity index (α_i) for the early (I–II) and late (IV) larval instars of *Anopheles stephensi* in the three predators, the mosquitofish *Gambusia affinis*, dragonfly naiads and copepod *Mesocyclops aspericornis*, offered the following combination of (*a*) early instars–*Daphnia similoides*, (*b*) early instars–*Moina macrocopa*, (*c*) late instars–*Daphnia similoides* and (*d*) late instars–*Moina macrocopa* in three different proportions. The dashed line indicates the value for non-selective feeding.

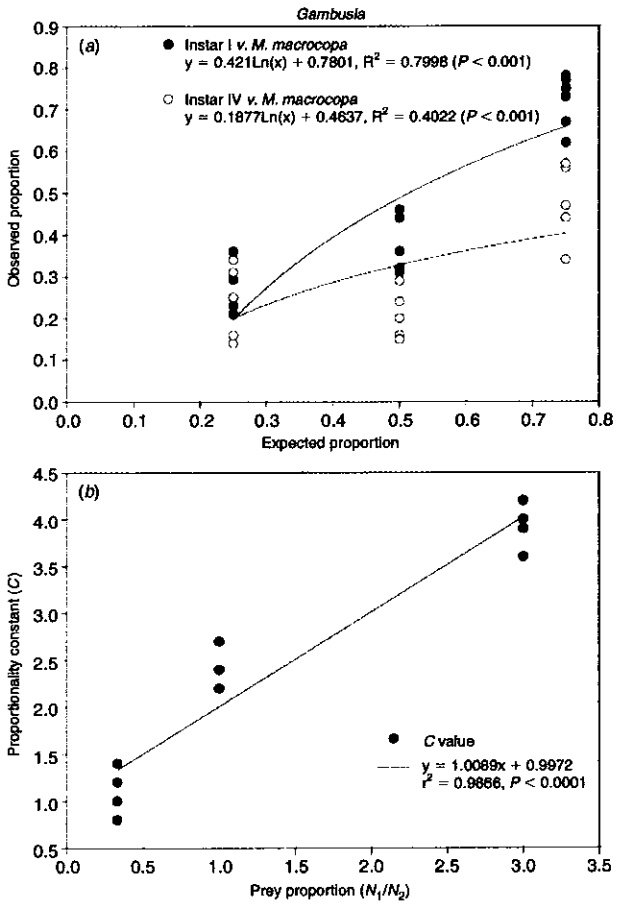

Fig. 8. (*a*) Prey switching in mosquitofish, in terms of observed *v.* expected prey proportion in the diet, offered either Instar-I or Instar-IV of *Anopheles stephensi* along with the cladoceran *Moina macrocopa*. (*b*) Value of *C* (i.e. proportionality constant: ratio of the proportion of prey in the diet and the initial proportion of prey in the medium) in relation to the initial ratio (N_1/N_2) of the two prey abundance in the medium offered a choice of Instar-I along with *Moina macrocopa*. Regression lines are drawn using the equations shown.

(i.e. the presence of alternative prey and different relative prey proportions do not affect a predator's preference for mosquito larvae).

The aim of the present investigation was to compare the potential usefulness of the three predators for mosquito control in natural conditions. The coefficient of determination (r^2) between the number consumed and larval size is 0.93, 0.78 and 0.89 for the mosquitofish, naiads, and copepods respectively. This means that 93%, 78% and 89% of the total variation (reduction of consumption) can be explained by larval size alone. Looking at the correlation of dry mass of larvae utilised by the predator *v.* larval size (Fig. 9), larger prey (late instars) turn out to be more profitable food for all predators than smaller instars.

Discussion

Conventional biological control programs have come under increasing scrutiny for their non-target effects (Howarth 1991; Hawkins and Marino 1997) and failure of candidate agents to perform in the field as expected on the basis of laboratory results (Murdoch *et al.* 1985; Hoelmer and Kirk 2005; Kumar and Hwang 2006). Taking into account both the risks associated with biological control, such as faunal introductions, and the impact of the controlling agent on non-target fauna, it is suggested that the selection of natural predators should be based on their foraging efficiency, behaviour and adaptation to the habitat of the targeted pests (Washburn 1995). In our consumption experiments, the mosquitofish consumed by far the highest number of early-instar mosquito larvae, followed by naiads and then copepods. However, the consumption of middle and late instars by the mosquitofish and naiads was comparable.

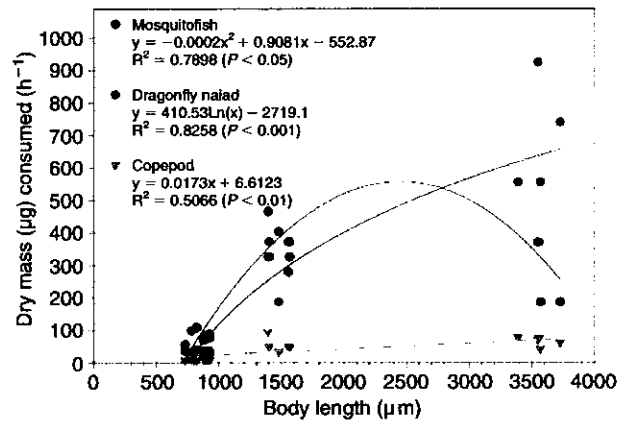

Fig. 9. Dry mass (µg) consumption rates in mosquitofish, naiads and copepods as a function of larval size of *Anopheles stephensi*. The trend lines were drawn using the equations shown.

From a pest-control perspective, an evaluation of the numbers of prey killed (but not necessarily eaten) is important. For the copepod *Mesocyclops aspericornis*, regardless of instar stage, wasteful killings were recorded in all trials. Copepods normally kill larger numbers of mosquito larvae than they actually use (Kumar and Rao 2003; Kumar and Hwang 2006; Marten and Reid 2007). Nonetheless, large species of cyclopoid copepods are common and they substantially reduce larval survival, or even eliminate mosquito production completely, wherever they occur. In an earlier study, Kumar and Rao (1999*a*) observed a negative correlation between prey size and the number of prey consumed by *Mesocyclops thermocyclopoides*. Higher energy gain from late instars of immature mosquitos in the mosquitofish and naiads indicates that these predators might prefer late instars to early instars in natural ecosystems where different instars are present concurrently. In such cases, a lower number of larvae will be required for the energy demand of the predators. The copepod used in the present study prefers early instars over late instars in both natural and laboratory conditions (Kumar and Rao 2003; Marten and Reid 2007).

Bence and Murdoch (1986) discovered that prey size alone does not determine preference in mosquitofish when its diet choice includes prey species differing in other attributes. Many foraging studies suggest a high individual specialisation, that is, an opportunistic feeding strategy for mosquitofish in both the

juvenile and adult stages (Swanson *et al.* 1996; Specziar 2004). As in our laboratory results, the preference for mosquito larvae by mosquitofish was dependent on prey type and its proportion in the medium. Under field conditions, dietary composition in the mosquitofish also changes with the relative abundance of prey in the habitat (Oaten and Murdoch 1975; Cabral *et al.* 1998; Willems *et al.* 2005). Larger-sized *Daphnia* was not a preferred food item for mosquitofish (Hurlbert and Mulla 1981; Bence and Murdoch 1986). In the present study, the basic selectivity patterns of the mosquitofish, notwithstanding the ability to switch prey preferences based on the relative abundance of the prey species, suggest that in nature mosquitofish are capable of exploiting their food resources optimally. Mosquitofish are able to attack a variety of prey and their preference might be determined by several factors, such as prey size and biomass (Wurtsbaugh *et al.* 1980; Hagman and Shine 2007), transparency, relative abundance of prey in the environment, and the swimming behaviour of the prey (Linden and Cech 1990; Swanson *et al.* 1996). Switching between different cladoceran prey, depending on their relative concentration in the medium, has been recorded for some carp and catfishes (Mookerji and Rao 1995).

In a field study on the eastern mosquitofish, *Gambusia holbrooki*, of Lake Banyoles, Catalonia, Spain, it was observed that its diet was based on littoral cladocerans, particularly *Chydorus sphaericus*, *Scapholeberis ramneri*, *Ceriodaphnia reticulata* and *Pleuroxus laevis*, and nematoceran (mainly chironomid) adults. There was a large variety of prey of terrestrial (collembolans and ants) or aquatic neustonic origin, which shows that the foraging range of microhabitats of the mosquitofish is closely linked to the water surface (Cabral and Marques 1999; Margaritora *et al.* 2001). *Gambusia* has a voracious appetite, feeding at rates much higher than those of most other similar-sized fish (Ivantsoff and Aarn 1999). At optimal temperatures (30–35°C), maximum consumption rates range from 0.75 to 1.1% of bodyweight day^{-1} for fish ranging from 0.2 to 1.2 g (Cabral *et al.* 1998). Our results differ from an earlier 24-h field study in which no significant difference was found in the quantity of first, second and third instar larvae consumed by mosquitofish (Willems *et al.* 2005). However, in that study they did record a significant negative relationship between the predation rate and larval instars (Willems *et al.* 2005), similar to our study.

Although mosquitofish have often been released in highly disturbed or artificial habitats, they have the potential to spread into pristine water bodies (Arthington and Lloyd 1989; Courtnay and Maffe 1989; Allen *et al.* 2002), where they can have a severe impact on native fishes (Howe *et al.* 1997; Gill *et al.* 1999; Nagdali and Gupta 2002), amphibians (Morgan and Buttemer 1996; Webb and Joss 1997; Goodsell and Kats 1999) and invertebrates (Leyse *et al.* 2004). Comparative research on introduced and native larvivorous fish feeding is scarce, although it is crucial to determine the impacts of their introduction to ecosystems (Willems *et al.* 2005). One comparative study of *G. holbrooki* and a common native species in eastern Australia, the Pacific Blue-eye, *Pseudomugil signifer* Kner, has suggested that the native fish *P. signifer* is potentially an effective predator of mosquito larvae (Durre 1991). In contrast, the mosquitofish has become a threat to native fishes that share similar habitats, particularly to cyprinodontids, because of its ecological advantages related to fast growth, early maturity, viviparity and its voracious appetite (Lloyd *et al.* 1986; Vargas and de Sostoa 1996).

Dragonflies and damselflies are natural predators of mosquitoes. Aquatic naiads use mosquito larvae as food, while the adults are efficient predators of airborne adult mosquitoes (Corbet 1980; Kumar and Hwang 2006). They also prey on zooplankton, midges, small moths, small tadpoles (Travis *et al.* 1985), beetles (Lundkvist *et al.* 2003), aquatic mite larvae (R. Kumar unpubl. data) and other insects (Brendonck *et al.* 2002). Contrary to these findings, Breene *et al.* (1990) did not observe any mosquito larvae in the gut of damselfly *Enallagma civile* naiads, although mosquito larvae (belonging to the genera *Aedes*, *Culex*, *Culiseta*, *Mansonia* and *Psorophora*) were observed in the pond from where the naiads were collected. The results obtained in the present study further support that dragonfly naiads do not prefer mosquito larvae in the presence of alternative natural prey.

In the present study, considerable reductions in the consumption of early instars of mosquito larvae in the presence of alternative prey do not imply control potential by naiads under natural conditions. However, an increased consumption of late instars in the presence of the larger cladoceran *Daphnia similoides* indicates that this species can consume late instars at a higher rate if less-preferred foods are available in the medium. Because of the typical and continuous swimming behaviour of *Daphnia*, these larvae are active and easily detectable by the odonate naiads, which facilitates a higher encounter of late instars in the presence of *Daphnia*. In aquatic ecosystems, the importance of ecological facilitation (Simberloff and Van Holle 1999; Stachowicz 2001) has been recorded, which suggests that by improving dispersal, increasing access to resources and providing refugium from stress, an organism profits from the presence of another (Cardinale *et al.* 2002; Bruno *et al.* 2003).

In an earlier study, odonate naiads disproportionately tended to crop the largest mosquito larvae available in the habitat (Fincke *et al.* 1997). Some features of naiads (e.g. larvivory, the ability to cling to the sides of a container so that they are not lost when water is removed, slower developing stages with no need of frequent replacement, extraordinary starvation tolerance) qualify them to be suitable candidates for control agents in different mosquito-breeding habitats in the absence of alternative food. However, as most odonates frequent the sides and bottom of a water body, they might have a greater impact on benthic prey than on mosquito larvae such as *Anopheles* or *Culex* that feed near the water surface (Fincke *et al.* 1997).

Cyclopoid copepods like *M. aspericornis* have been found to prefer mosquito larvae to a range of other prey (Kumar and Hwang 2006; Marten and Reid 2007). This copepod showed a higher preference for early larval instars when *D. similoides* was present as alternative prey. In our earlier study, the number of prey eliminated by the copepod *M. thermocyclopoides* increased with increasing prey density (Kumar and Rao 2003). The per capita larval removal by the copepod *M. aspericornis* in the present study is comparable to that recorded by Brown *et al.* (1991*a*), Marten *et al.* (1994*a*) and Kumar and Rao (2003). Shallow and eutrophic water bodies that are also breeding sites for mosquitoes are often inhabited by this cyclopoid copepod. Higher densities of mosquito larvae are common during the warmer months of the year. Along with other zooplankton

species (ciliates, rotifers and cladocerans) the mosquito larvae provide potential food sources for this predominantly predatory copepod. Field and laboratory studies have shown that many species of *Mesocyclops* feed efficiently on the larvae of *Anopheles* and *Aedes* (Marten *et al.* 1989, 1994*b*; Marten 1990; Kay 1996). In the field, a strong negative correlation has been reported between populations of copepod representatives, such as *Mesocyclops* spp., and *Anopheles albimanus* larvae (Marten *et al.* 1989; Rey *et al.* 2004), indicating the suitability of this copepod as a biological control agent for vectorial mosquitoes (Brown *et al.* 1991*a*, 1991*b*; Andrealis and Gere 1992; Jennings *et al.* 1995; Kay *et al.* 2000). Although capable of feeding on a wide spectrum of prey species, cyclopoids are rather selective in their prey (Janicki and Decosta 1990; Rao and Kumar 2002).

The larvivorous efficiency of the cyclopoid *M. aspericornis* was clearly shown in our study. This efficiency, coupled with the copepod's frequent occurrence in fairly high ($\sim$400 L^{-1}) densities in shallow, eutrophic water bodies, indicates its suitability in controlling mosquito populations. The efficacy of *Mesocyclops* in suppressing larval populations of *Aedes aegypti* has been demonstrated in field trials in Louisiana (Marten *et al.* 1989, 1994*b*), Vietnam (Nam *et al.* 1998) and Venezuela (Zoppi de Roa *et al.* 2002). *Mesocyclops aspericornis* is particularly promising as an efficient biological agent for controlling vectorial species of mosquitoes (such as *A. stephensi*) in urban stagnant waters because it thrives in small, stagnant water bodies that are potential mosquito breeding sites that are too small for stocking larvivorous fish, such as *Gambusia* spp., but that can support desirable levels of adult copepod populations. Freshly emerged adult females have enough endogenous energy reserves to survive for up to 30 days without any external food supply (Kumar and Rao 1999*b*). The ability of the copepods to enter a dormant stage helps them to survive in small temporary waterbodies that dry up periodically (Williams-Howze 1997; Brendonck *et al.* 2002).

The selectivity patterns in all three predators that we observed in the present study can be explained in terms of the energetic costs of prey capture. We observed that copepods had to strike repeatedly on the middle part of the larval body to subdue it, and that there was an $\sim$20% probability of a captured larva escaping by forceful wriggling. Marten *et al.* (1994*a*) noted the role of the bristles and thick cuticle of *Culex* larvae in reducing vulnerability to the larvivorous copepods *Mesocyclops albidus* and *Mesocyclops longisetus*. For mosquitofish and naiads, all larval instars were successfully captured if attacked. We found ejection of late instar mosquito larvae, however, after capture. Although consumption of mosquito larvae by mosquitofish was higher than that by other predators in the present study, their selectivity index did not differ significantly from that of the copepod.

Our results strongly advocate that a predator's selection patterns for the target species should always be considered before release of a control agent into a natural setting, where wide ranges of foods are available (Barbosa and Segarra-Carmona 1993; Kumar and Rao 2003; Hoelmer and Kirk 2005). Our observation of the patterns of prey preference in the three larvicidal animals commonly thought to be control agents of mosquito larvae highlights the following aspects. First, unlike the copepod neither mosquitofish nor naiads showed a consistent preference, not even for the early instars of mosquitoes. Second, dragonfly naiads consistently avoided mosquito larvae (except at one proportion in the early instar–*D. similoides* choice test), regardless of prey type and their relative abundance in the environment. The preference for larvae demonstrated by the mosquitofish was largely a function of the type of alternative prey and their relative abundances in our laboratory microcosm. The predators used in these predation trials displayed a variety of behavioural characteristics and they can be ranked along a gradient of average swimming speed as copepod < naiad < mosquitofish. The mosquitofish swims more or less continuously (Cooper *et al.* 1985), the copepod (*Mesocyclops* used in the present study) also moves continuously and pounces on prey vigorously in response to any hydrodynamic disturbance (Williamson 1983; Rao and Kumar 2002; Kumar 2003), while naiads demonstrate rapid attack acceleration in their swimming speed (Pritchard 1965; the present study). Prey that are less active and patchily distributed in space and time (e.g. mosquito larvae) are more likely to be discovered and eaten by actively foraging predators (Huey and Pianka 1981; Krebs and Kacelnik 1991). The differential preference of predators for mosquito larvae can be seen in light of their behavioural attributes and microhabitats. The mosquitofish is a pelagic predator normally preying near the water surface, the copepod preys throughout the water column and the naiads cling to the bottom or sides of the water body and ambush their prey.

Because of their invasiveness, dispersal patterns and generalistic foraging behaviour, *G. affinis* and *G. holbrooki* (Girard, 1859) have been designated among the world's 100 most invasive species (ISSG 2000). The negative impacts of mosquitofish on native communities have been well established. Ritchie and Laidlaw-Bell (1994) found that ovipositing *Aedes taeniorhynchus* (Wiedemann, 1821) strongly avoided sites with high densities of mosquitofish and moved to nearby habitats with few or no predatory fish. Similar results have been obtained by other workers (Stav *et al.* 2000). Therefore, adult female mosquitoes often avoid ovipositing in waters containing predatory fish (Angelon and Petranka 2002; Kiflawi *et al.* 2003). This suggests that the effectiveness of *Gambusia* in controlling mosquitoes might be compromised if adult mosquitoes avoid waters that are stocked with fish by shifting to nearby breeding sites that lack fish. The feasibility of using copepods in operational control programs remains to be evaluated at a large scale. Rivière *et al.* (1987*a*, 1987*b*) conducted the first large-scale field trials by introducing the copepod *M. aspericornis* to crabholes, a breeding place for *Aedes polynesiensis* (Marks, 1951) and *Aedes aegypti* (Meigen, 1818) in Tahiti. Whenever present, *M. aspericornis* reduced larval survival by 91–99% in the field. However, there was no significant long-term effect because the copepods failed to survive when the crab holes dried out. Lardeux *et al.* (1992) introduced *M. aspericornis* to all water storage tanks and 200-L drums in a village in French Polynesia. Populations of *A. aegypti* was suppressed in water storage containers where *M. aspericornis* established a population. Kay *et al.* (2002*a*) surveyed service manholes and pits, breeding habitats of *Ochlerotatus* and *Aedes* mosquitoes in northern Queensland storm drains, and recorded a strong negative association between the presence of *Mesocyclops* spp. (presumably *M. aspericornis* and *M. darwini*) and the presence of *Ochlerotatus* or *Aedes* larvae. The subsequent introduction

of *Mesocyclops* to storm drains demonstrated the effectiveness of the copepods for mosquito control (Kay *et al.* 2002*a*). Fifty individuals of *Mesocyclops* were introduced to a single service manhole in Townsville, Queensland. All manholes in the vicinity were monitored for 1 year before the *Mesocyclops* introduction and for 3 years afterwards. *Mesocyclops* had spread to manholes as far away as 2 km from the introduction site by the year after introduction, reaching 83% of the manholes in an area of 1.3 km^2 by the third year. Once in a manhole, the copepods established populations for many years. Individuals were not washed out of the manholes by high water flows, and they survived in the damp sediment during dry periods. Over the entire monitoring period, 11% of the manhole inspections without *Mesocyclops* were positive for *Ochlerotatus* or *Aedes* larvae. In contrast, only 3% of the inspections of manholes with *Mesocyclops* revealed any larvae at all. The absolute impact of copepods (Kay *et al.* 2002*b*) on the production of *Ochlerotatus* and *Aedes* became particularly clear when 50 *M. aspericornis* and *M. darwini* were introduced into four storm drain service pits where thousands of larvae had been found. Within 4–6 months, the number of *Ochlerotatus* and *Aedes* larvae had declined to zero.

The differential preference patterns for larval mosquito feeding modes, and the microdistribution and diurnal feeding rhythm of the three predators used in the present study raise further questions. For example, can these predators be used for control purposes in a multiple predator control system and can they be introduced concurrently to mosquito-breeding habitats. As an integrated pest-control program, multiple predators and/or the use of microbes as well as predators can be tested for their feasibility to achieve an acceptable range of control in natural conditions (Lardeux *et al.* 2002; Chansang *et al.* 2004; Kay and Nam 2005). It has been shown that mosquitofish prefer neither copepods nor naiads as food items (Pritchard 1965; Cooper *et al.* 1985). In contrast, naiads are relatively efficient at capturing copepods (Cooper *et al.* 1985). Cannibalism has been reported in all three predators used in the present study and we observed cannibalism in our controls where prey were absent. In earlier studies (Kumar 1981; Kumar and Rao 1999*a*), cannibalism was not recorded in the presence of even less-preferred prey for naiads and copepods, but occurred in the absence of alternative food.

The use of multiple predators, for example, fish and copepods or fish and naiads, needs to be evaluated. It is important to note that fish are visual predators, whereas copepods and naiads are tactile predators. Therefore, fish-imposed control will only be functional during daylight hours. Naiads actively feed during twilight, whereas copepods possess equal killing efficiency during day and night using hydromechanical signals (Hwang and Strickler 2001). Copepods can kill larval instars in thick aquatic vegetation, where larvae can hide from fish (Lindberg 1949; Laird 1988). The temporary nature of the aquatic phase eliminates fish and allows invertebrates to become major biotic determinants that regulate community structure. Biological control agents should be evaluated with respect to their climatic compatibility and their capability to maintain very close interactions with target populations (Murdoch *et al.* 1985; Waage and Greathead 1988). Another positive attribute of copepods as control agents is that the ovipositing mosquito females do not avoid water where copepods are present. Rather, copepod-conditioned water or the presence of copepods acted as an attractant for gravid *Aedes aegypti* females (Torres-Estrada *et al.* 2001).

As natural communities usually include an array of predators, there are two questions related to the integrated vector control program that are yet to be solved. The first question is: does the use of multiple predators synergistically increase the rate of mosquito larval removal? None of the predators is strictly monophagous and they might be competing for the same prey. Therefore, it is critical to understand the effects that multiple predators might have on each other and on their shared prey populations. The second question is: do multiple agents actually reduce the likelihood of success through the process of competitive exclusion or intraguild predation (Rosenheim *et al.* 1995)? Both these questions are priorities for future research in this important area of pest control in aquatic ecosystems.

Acknowledgements

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juvenile and adult stages (Swanson *et al.* 1996; Specziar 2004). As in our laboratory results, the preference for mosquito larvae by mosquitofish was dependent on prey type and its proportion in the medium. Under field conditions, dietary composition in the mosquitofish also changes with the relative abundance of prey in the habitat (Oaten and Murdoch 1975; Cabral *et al.* 1998; Willems *et al.* 2005). Larger-sized *Daphnia* was not a preferred food item for mosquitofish (Hurlbert and Mulla 1981; Bence and Murdoch 1986). In the present study, the basic selectivity patterns of the mosquitofish, notwithstanding the ability to switch prey preferences based on the relative abundance of the prey species, suggest that in nature mosquitofish are capable of exploiting their food resources optimally. Mosquitofish are able to attack a variety of prey and their preference might be determined by several factors, such as prey size and biomass (Wurtsbaugh *et al.* 1980; Hagman and Shine 2007), transrelated to fast growth, early maturity, viviparity and its voracious appetite (Lloyd *et al.* 1986; Vargas and de Sostoa 1996).

Dragonflies and damselflies are natural predators of mosquitoes. Aquatic naiads use mosquito larvae as food, while the adults are efficient predators of airborne adult mosquitoes (Corbet 1980; Kumar and Hwang 2006). They also prey on zooplankton, midges, small moths, small tadpoles (Travis *et al.* 1985), beetles (Lundkvist *et al.* 2003), aquatic mite larvae (R. Kumar unpubl. data) and other insects (Brendonck *et al.* 2002). Contrary to these findings, Breene *et al.* (1990) did not observe any mosquito larvae in the gut of damselfly *Enallagma civile* naiads, although mosquito larvae (belonging to the genera *Aedes*, *Culex*, *Culiseta*, *Mansonia* and *Psorophora*) were observed in the pond from where the naiads were collected. The results obtained in the present study further support that dragonfly naiads do not prefer mosquito larvae in the presence of

species (ciliates, rotifers and cladocerans) the mosquito larvae provide potential food sources for this predominantly predatory copepod. Field and laboratory studies have shown that many species of *Mesocyclops* feed efficiently on the larvae of *Anopheles* and *Aedes* (Marten *et al.* 1989, 1994*b*; Marten 1990; Kay 1996). In the field, a strong negative correlation has been reported between populations of copepod representatives, such as *Mesocyclops* spp., and *Anopheles albimanus* larvae (Marten *et al.* 1989; Rey *et al.* 2004), indicating the suitability of this copepod as a biological control agent for vectorial mosquitoes (Brown *et al.* 1991*a*, 1991*b*; Andrealis and Gere 1992; Jennings *et al.* 1995; Kay *et al.* 2000). Although capable of feeding on a wide spectrum of prey species, cyclopoids are rather selective in their prey (Janicki and Decosta 1990; Rao and Kumar 2002).

The larvivorous efficiency of the cyclopoid *M. aspericornis* was clearly shown in our study. This efficiency, coupled with the copepod's frequent occurrence in fairly high ($\sim 400\,L^{-1}$) densities in shallow, eutrophic water bodies, indicates its suitability in controlling mosquito populations. The efficacy of *Mesocyclops* in suppressing larval populations of *Aedes aegypti* has been demonstrated in field trials in Louisiana (Marten *et al.* 1989, 1994*b*), Vietnam (Nam *et al.* 1998) and Venezuela (Zoppi de Roa *et al.* 2002). *Mesocyclops aspericornis* is particularly promising as an efficient biological agent for controlling vectorial species of mosquitoes (such as *A. stephensi*) in urban stagnant waters because it thrives in small, stagnant water bodies that are potential mosquito breeding sites that are too small for stocking larvivorous fish, such as *Gambusia* spp., but that can support desirable levels of adult copepod populations. Freshly emerged adult females have enough endogenous energy reserves to survive for up to 30 days without any external food supply (Kumar and Rao 1999*b*). The ability of the copepods to enter a dormant stage helps them to survive in small temporary waterbodies that dry up periodically (Williams-Howze 1997; Brendonck *et al.* 2002).

The selectivity patterns in all three predators that we observed in the present study can be explained in terms of the energetic costs of prey capture. We observed that copepods had to strike repeatedly on the middle part of the larval body to subdue it, and that there was an ~20% probability of a captured larva escaping by forceful wriggling. Marten *et al.* (1994*a*) noted the role of the bristles and thick cuticle of *Culex* larvae in reducing vulnerability to the larvivorous copepods *Mesocyclops albidus* and *Mesocyclops longisetus*. For mosquitofish and naiads, all larval instars were successfully captured if attacked. We found ejection of late instar mosquito larvae, however, after capture. Although consumption of mosquito larvae by mosquitofish was higher than that by other predators in the present study, their selectivity index did not differ significantly from that of the copepod.

Our results strongly advocate that a predator's selection patterns for the target species should always be considered before release of a control agent into a natural setting, where wide ranges of foods are available (Barbosa and Segarra-Carmona 1993; Kumar and Rao 2003; Hoelmer and Kirk 2005). Our observation of the patterns of prey preference in the three larvicidal animals commonly thought to be control agents of mosquito larvae highlights the following aspects. First, unlike the copepod neither mosquitofish nor naiads showed a consistent preference, not even for the early instars of mosquitoes. Second, dragonfly naiads consistently avoided mosquito larvae (except at one proportion in the early instar–*D. similoides* choice test), regardless of prey type and their relative abundance in the environment. The preference for larvae demonstrated by the mosquitofish was largely a function of the type of alternative prey and their relative abundances in our laboratory microcosm. The predators used in these predation trials displayed a variety of behavioural characteristics and they can be ranked along a gradient of average swimming speed as copepod < naiad < mosquitofish. The mosquitofish swims more or less continuously (Cooper *et al.* 1985), the copepod (*Mesocyclops* used in the present study) also moves continuously and pounces on prey vigorously in response to any hydrodynamic disturbance (Williamson 1983; Rao and Kumar 2002; Kumar 2003), while naiads demonstrate rapid attack acceleration in their swimming speed (Pritchard 1965; the present study). Prey that are less active and patchily distributed in space and time (e.g. mosquito larvae) are more likely to be discovered and eaten by actively foraging predators (Huey and Pianka 1981; Krebs and Kacelnik 1991). The differential preference of predators for mosquito larvae can be seen in light of their behavioural attributes and microhabitats. The mosquitofish is a pelagic predator normally preying near the water surface, the copepod preys throughout the water column and the naiads cling to the bottom or sides of the water body and ambush their prey.

Because of their invasiveness, dispersal patterns and generalistic foraging behaviour, *G. affinis* and *G. holbrooki* (Girard, 1859) have been designated among the world's 100 most invasive species (ISSG 2000). The negative impacts of mosquitofish on native communities have been well established. Ritchie and Laidlaw-Bell (1994) found that ovipositing *Aedes taeniorhynchus* (Wiedemann, 1821) strongly avoided sites with high densities of mosquitofish and moved to nearby habitats with few or no predatory fish. Similar results have been obtained by other workers (Stav *et al.* 2000). Therefore, adult female mosquitoes often avoid ovipositing in waters containing predatory fish (Angelon and Petranka 2002; Kiflawi *et al.* 2003). This suggests that the effectiveness of *Gambusia* in controlling mosquitoes might be compromised if adult mosquitoes avoid waters that are stocked with fish by shifting to nearby breeding sites that lack fish. The feasibility of using copepods in operational control programs remains to be evaluated at a large scale. Rivière *et al.* (1987*a*, 1987*b*) conducted the first large-scale field trials by introducing the copepod *M. aspericornis* to crabholes, a breeding place for *Aedes polynesiensis* (Marks, 1951) and *Aedes aegypti* (Meigen, 1818) in Tahiti. Whenever present, *M. aspericornis* reduced larval survival by 91–99% in the field. However, there was no significant long-term effect because the copepods failed to survive when the crab holes dried out. Lardeux *et al.* (1992) introduced *M. aspericornis* to all water storage tanks and 200-L drums in a village in French Polynesia. Populations of *A. aegypti* was suppressed in water storage containers where *M. aspericornis* established a population. Kay *et al.* (2002*a*) surveyed service manholes and pits, breeding habitats of *Ochlerotatus* and *Aedes* mosquitoes in northern Queensland storm drains, and recorded a strong negative association between the presence of *Mesocyclops* spp. (presumably *M. aspericornis* and *M. darwini*) and the presence of *Ochlerotatus* or *Aedes* larvae. The subsequent introduction

of *Mesocyclops* to storm drains demonstrated the effectiveness of the copepods for mosquito control (Kay *et al.* 2002*a*). Fifty individuals of *Mesocyclops* were introduced to a single service manhole in Townsville, Queensland. All manholes in the vicinity were monitored for 1 year before the *Mesocyclops* introduction and for 3 years afterwards. *Mesocyclops* had spread to manholes as far away as 2 km from the introduction site by the year after introduction, reaching 83% of the manholes in an area of 1.3 km^2 by the third year. Once in a manhole, the copepods established populations for many years. Individuals were not washed out of the manholes by high water flows, and they survived in the damp sediment during dry periods. Over the entire monitoring period, 11% of the manhole inspections without *Mesocyclops* were positive for *Ochlerotatus* or *Aedes* larvae. In contrast, only 3% of the inspections of manholes with *Mesocyclops* revealed any larvae at all. The absolute impact of copepods (Kay *et al.* 2002*b*) on the production of *Ochlerotatus* and *Aedes* became particularly clear when 50 *M. aspericornis* and *M. darwini* were introduced into four storm drain service pits where thousands of larvae had been found. Within 4–6 months, the number of *Ochlerotatus* and *Aedes* larvae had declined to zero.

The differential preference patterns for larval mosquito feeding modes, and the microdistribution and diurnal feeding rhythm of the three predators used in the present study raise further questions. For example, can these predators be used for control purposes in a multiple predator control system and can they be introduced concurrently to mosquito-breeding habitats. As an integrated pest-control program, multiple predators and/or the use of microbes as well as predators can be tested for their feasibility to achieve an acceptable range of control in natural conditions (Lardeux *et al.* 2002; Chansang *et al.* 2004; Kay and Nam 2005). It has been shown that mosquitofish prefer neither copepods nor naiads as food items (Pritchard 1965; Cooper *et al.* 1985). In contrast, naiads are relatively efficient at capturing copepods (Cooper *et al.* 1985). Cannibalism has been reported in all three predators used in the present study and we observed cannibalism in our controls where prey were absent. In earlier studies (Kumar 1981; Kumar and Rao 1999*a*), cannibalism was not recorded in the presence of even less-preferred prey for naiads and copepods, but occurred in the absence of alternative food.

The use of multiple predators, for example, fish and copepods or fish and naiads, needs to be evaluated. It is important to note that fish are visual predators, whereas copepods and naiads are tactile predators. Therefore, fish-imposed control will only be functional during daylight hours. Naiads actively feed during twilight, whereas copepods possess equal killing efficiency during day and night using hydromechanical signals (Hwang and Strickler 2001). Copepods can kill larval instars in thick aquatic vegetation, where larvae can hide from fish (Lindberg 1949; Laird 1988). The temporary nature of the aquatic phase eliminates fish and allows invertebrates to become major biotic determinants that regulate community structure. Biological control agents should be evaluated with respect to their climatic compatibility and their capability to maintain very close interactions with target populations (Murdoch *et al.* 1985; Waage and Greathead 1988). Another positive attribute of copepods as control agents is that the ovipositing mosquito females do not avoid water where copepods are present. Rather, copepod-conditioned water or the presence of copepods acted as an attractant for gravid *Aedes aegypti* females (Torres-Estrada *et al.* 2001).

As natural communities usually include an array of predators, there are two questions related to the integrated vector control program that are yet to be solved. The first question is: does the use of multiple predators synergistically increase the rate of mosquito larval removal? None of the predators is strictly monophagous and they might be competing for the same prey. Therefore, it is critical to understand the effects that multiple predators might have on each other and on their shared prey populations. The second question is: do multiple agents actually reduce the likelihood of success through the process of competitive exclusion or intraguild predation (Rosenheim *et al.* 1995)? Both these questions are priorities for future research in this important area of pest control in aquatic ecosystems.

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BACILLUS THURINGIENSIS SEROVARIETY *ISRAELENSIS* AND *BACILLUS SPHAERICUS* FOR MOSQUITO CONTROL

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KEY WORDS *Bacillus thuringiensis* serovar. *israelensis (Bti)*, *Bacillus sphaericus*, binary toxin, factors affecting efficacy, resistance, mode of action, structure, safety, ecotoxicology, integrated mosquito control

ABSTRACT. Since the discovery of *Bacillus thuringiensis* (Berliner) serovariety *israelensis* de Barjac (*Bti*) and efficacious isolates of *Bacillus sphaericus* Neide, formulations of these bacteria have become the predominant non-chemical means employed for control of mosquito larvae at several locations in the United States and other countries. An overview of developments in the past 20 years is presented in this chapter regarding the toxins of *Bti* and *B. sphaericus*, their modes of action, efficacy and factors that affect larvicidal activity, development of resistance, safety, and their roles in integrated mosquito control. The efficacy of *Bti* formulations has been demonstrated in a variety of habitats against a multitude of species of mosquitoes. *B. sphaericus* formulations have been utilized predominantly in organically enriched habitats against *Culex* species, but they are also active in a variety of habitats having low organic enrichment, against numerous species, and across several genera. *Stegomyia* spp. are not susceptible to practical doses of *B. sphaericus* formulations. *B. sphaericus* has been shown to persist longer than *Bti* in polluted habitats and, under certain circumstances, can recycle in larval cadavers. A disadvantage of *B. sphaericus* has been the development of resistance in certain populations of *Cx. quinquefasciatus* Say and *Cx. pipiens* Linnaeus. Biotic and abiotic factors that influence the larvicidal activity of *Bti* and *B. sphaericus* include species of mosquito and their respective feeding strategies, rate of ingestion, age and density of larvae, habitat factors (temperature, solar radiation, depth of water, turbidity, tannin and organic content, presence of vegetation, etc.), formulation factors (type of formulation, toxin content, how effectively the material reaches the target, and settling rate), storage conditions, production factors, means of application and frequency of treatments. Due to their efficacy and relative specificity, both *Bti* and *B. sphaericus* can be ideal control agents in integrated programs especially where other biological control agents, environmental management, personal protection and the judicious use of insecticides are combined.

INTRODUCTION

Bacteria received limited attention as microbial control agents of mosquitoes before the discovery of *Bacillus thuringiensis* (Berliner) serovariety *israelensis* de Barjac (*Bti*) and efficacious strains of *Bacillus sphaericus* Neide. Today, formulations of *Bti* are the predominant non-chemical means employed for control of mosquito larvae in the United States and several other countries. At the time the original *Biological Control of Mosquitoes* (Chapman, 1985) was published, a substantial number of laboratory studies and field evaluations of *Bti* had already been reported (Lacey, 1985a). Progress had also been made at that time on the study and evaluation of *B. sphaericus* (Singer, 1985), although commercial development lagged behind that of *Bti*. Because the number of publications on these 2 bacteria since 1985 is astronomical, this chapter will not exhaustively review the literature, but will highlight the developments in the past 20 years regarding the larvicidal toxins, their modes of action, efficacy and factors that affect activity, development of resistance, safety, and the role of bacteria in integrated mosquito control. This chapter will not cover recombinant bacteria for mosquito control. For a more detailed presentation of this topic see Federici et al. (2003) and the chapter by Federici et al. in this volume.

BACILLUS THURINGIENSIS

GENERAL BACKGROUND

Bacillus thuringiensis (*Bt*) is a gram-positive, spore forming, aerobic bacterium found in a multitude of habitats. It is distinguished from closely related *Bacillus* species by the presence of a parasporal inclusion. *Bt* serovarieties with larvicidal activity for Lepidoptera, Coleoptera, Diptera, and other insects have been isolated from a diversity of habitats worldwide including dead insects, soil, the phylloplane, grain dust, aquatic and other habitats (Martin and Traver 1989, Smith and Couche 1991, Beegle and Yamamoto 1992, Meadows 1993, Damgaard 2000, Glare and O'Callaghan 2000). Several *Bt* isolates have been developed and marketed for insect control (Beegle and Yamamoto 1992). *Bt* products represent the majority of biopesticide sales in North America and elsewhere.

The larvicidal activity of *Bt* is due to the toxins found in the parasporal inclusions (also known as parasporal bodies and crystals) that are produced at the time of sporulation. Collectively referred to as δ-endotoxins (delta-endotoxins) they comprise a diverse group of proteinaceous toxins, inclusion shapes and host ranges. Comparison of the amino acid sequences and host ranges of the individual toxins resulted in the development of a new

nomenclature for the inclusion toxins (Höfte and Whiteley 1989). The nomenclature has since been revised and is now based solely on amino acid sequence relationships between the toxins (Crickmore et al. 1998) enabling correlations to be made between the sequence, larvicidal activity, and evolutionary origins of the toxins (Crickmore 2000). The designation "Cry" refers to crystal toxins and "Cyt" is utilized to describe those crystal toxins displaying cytolytic and hemolytic activity in vitro. For individual toxins, Cry or Cyt is followed by an alpha-numeric designation. Toxins with the highest mosquito larvicidal activity (Cry4A, Cry4B, Cry11A and Cyt1A) are found in *Bti* and certain isolates of other serovarieties that produce the same or similar toxins. For an explanation of how serovarieties are determined, see Thiery and Frachon (1997).

A broad range of mosquito larvicidal activity has been reported for serovarieties of *Bt* other than *Bti* (Hall et al. 1977, Padua et al. 1980, Lacey and Oldacre 1983, Padua et al. 1984, Yu et al. 1991, Ragni et al. 1996, Thiery et al. 1999 and several others reviewed by Delécluse et al. 2000), and isolates of *Clostridium bifermentans* serovar. *malaysia* (de Barjac et al. 1990), *Bacillus circulans* (Darriet and Hougard 2002), *Brevibacillus laterosporus* (Favret and Yousten 1985), and *B. sphaericus* (reviewed in detail in this chapter). The level of larvicidal activity of most of these isolates is lower than that of *Bti*. Notable exceptions are the PG-14 isolate of *Bt* serovar. *morrisoni* (Padua et al. 1984, Federici et al. 1987, Lacey et al. 1988a) and some of the isolates reported by Ragni et al. (1996). *Bt* isolates with mosquito larvicidal activity have been placed in 3 classes by Delécluse et al. (2000): Class 1 includes *Bti* and 7 additional strains with toxin polypeptides and larvicidal activity that is similar or identical to that found in *Bti*; Class 2 includes 2 strains that are nearly as toxic as *Bti*, but with different polypeptides; and Class 3 has weakly active strains and polypeptides that are different from *Bti* and the other highly toxic strains.

Discovery, Description, and Development

Bti was discovered in 1976 by Goldberg and Margalit (1977) and as the varietal name indicates was found in Israel, where it was isolated from dead *Culex pipiens* Linnaeus larvae in a small riverbed pond contaminated with organic matter in the north central Negev Desert (Margalit and Dean 1985). It was subsequently identified as a new serovariety (H-14) by de Barjac (1978b). Commercial development rapidly followed the discovery of *Bti* for a few principal reasons: It is highly efficacious for larvae of mosquitoes and black flies, and safe for the vast majority of nontarget organisms; several studies around the world attesting to this were encouraged and subsidized by the World Health Organization (WHO). Also it can be produced using artificial media, is stable under a range of storage conditions, and can be applied using conventional equipment. The first commercial products based on *Bti* were distributed in the United States: Vectobac®, Bactimos®, and Teknar® were produced by Abbott Laboratories, Biochem Ltd. and Sandoz, respectively. Formulations included wettable powders, a variety of granules, flowable concentrates, and slow-release tablets and briquettes. As of 2005, the major supplier of *Bti* in North America is Valent BioSciences USA. Bayer Research, Becker Microbial Products, and Summit Chemical also supply *Bti* products in North America.

The quantification of larvicidal activity of products has received continuing attention since *Bti* was commercialized. Protocols for laboratory bioassay of *Bti* technical powders and formulations have been presented by Sinègre et al. (1981), Dulmage et al. (1985), Lacey (1997), Skovmand and Becker (2000) and several others over the past 20 years. The potency of commercial products is usually based on comparison with standard reference powders. Many of the standards have been produced by the Pasteur Institute and are listed by Thiery and Hamon (1998). Dulmage et al. (1985) proposed a U.S. standard, but these have not been maintained. Skovmand et al. (1997, 1998, 2000b) discuss the various parameters influencing potency of *Bti* products and the inter-laboratory variations in conducting assays.

Like other *Bt* varieties, *Bti* produces a parasporal inclusion at the time of sporulation containing the larvicidal toxins (Fig. 1A). In *Bti* the inclusion is not crystalline in appearance as seen in most other serovarieties, but is somewhat spherical (Fig. 1B). The Cry4A (125 kDa), Cry4B (135 kDa), Cry11A (68 kDa). and Cyt1A (28 kDa) toxins of *Bti* that are responsible for its elevated larvicidal activity for mosquitoes are located in distinctly separate areas of the inclusion, giving it a variegated appearance (Figs. 1A–E). In addition to the 4 toxins listed above, Pérez et al. (2005) also include Cry 10Aa and Cyt2Ba. Berry et al. (2002) sequenced the entire toxin-encoding megaplasmid, pBtoxis. The toxins in *Bti* are also active against several other dipteran species in the sub-order Nematocera, including black flies (Simuliidae: Molloy 1982, Lacey and Undeen 1987, Guillet et al. 1990, Adler et al. 2004); chironomid midges (Chironomidae: Ali 1981, Mulla et al. 1990a, Rodcharoen et al. 1991, Hughes et al. 2005); fungus gnats (Sciaridae: Cantwell and Cantelo 1984, Grewal 2000); crane flies (Tipulidae: Waalwijk et al. 1992); filter flies (Psychodida: Houston et al. 1989); and certain species in other families of Nematocera (Boisvert and Boisvert 2000, Lacey and Merritt 2003).

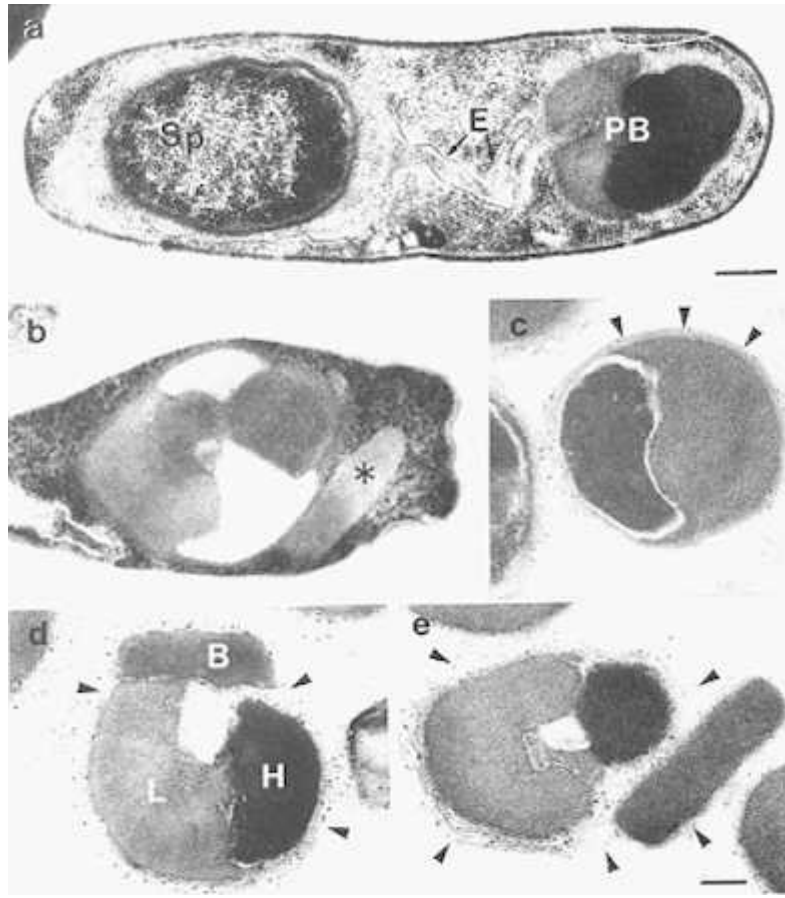

Fig. 1. Sporulating cell and parasporal bodies of *Bacillus thuringiensis* subsp. *israelensis*. a, Sporulating cell showing a developing spore (Sp) and parasporal body (PB). b, Fully developed parasporal body just prior to cell lysis. The asterisk identifies the Cry11A inclusion body, which is typically loosely attached to the parasporal body. c, Spherical parasporal body characteristic of *Bti*. The arrowheads point to the fibrous matrix of unknown composition that surrounds the inclusions, holding them together. d, Parasporal body illustrating inclusions of different electron densities. The bar-shaped inclusion contains Cry11A, whereas the low density inclusion (L) contains Cyt1A, and the high density inclusion (H) apparently contains both Cry4A and Cry4B. e, an inclusion similar to the one in (d) illustrating some of the variations that can occur in inclusion size and shape. E, exosporium membrane. Bar in (a) = 250 nm, and in (e) = 50 nm. Figure courtesy of Brian Federici.

Mode of Action and Structure of Larvicidal Toxins

The parasporal inclusions of *Bti* must be ingested in order to be active in susceptible species. The inclusions are solubilized in the alkaline midgut and cleaved to the larvicidal moieties by proteases. The lethal effect of *Bti* is due to the proteinaceous toxins binding to the surface membranes of the epithelial cells in the larval midgut and disrupting osmotic balance of the cells. The 3-domain structure of the Cry 4Ba toxin has recently been described by Boonserm et al. (2005) (Fig. 2). Although it is similar to that reported for the Cry 3A and Cry1Aa toxins, Boonserm et al. (2003, 2005) report some distinctions. The toxin components have 2 major functions related to larvicidal activity: receptor binding and membrane insertion. Domain 1 is believed to be the portion of the toxin that is inserted into the brush border membrane of midgut cells. This insertion results in the formation of channels and loss of osmotic balance and ultimately in the lysis of the cell (Schwartz and Laprade 2000, Boonserm et al. 2005). Domain 2 contains a receptor-binding region and is believed to determine insect specificity. Domain 3 also has a receptor-binding site and contributes to insect specificity. Details on the molecular mode of action of *Bti* Cry toxins are presented by Thomas and Ellar (1983), Crickmore et al. (1995), Schwartz and Laprade (2000), de Maagd et al. (2003), Boonserm et al. (2005), Fernandez et al. (2005), Pérez et al. (2005), and in the chapter by Federici et al. (this volume).

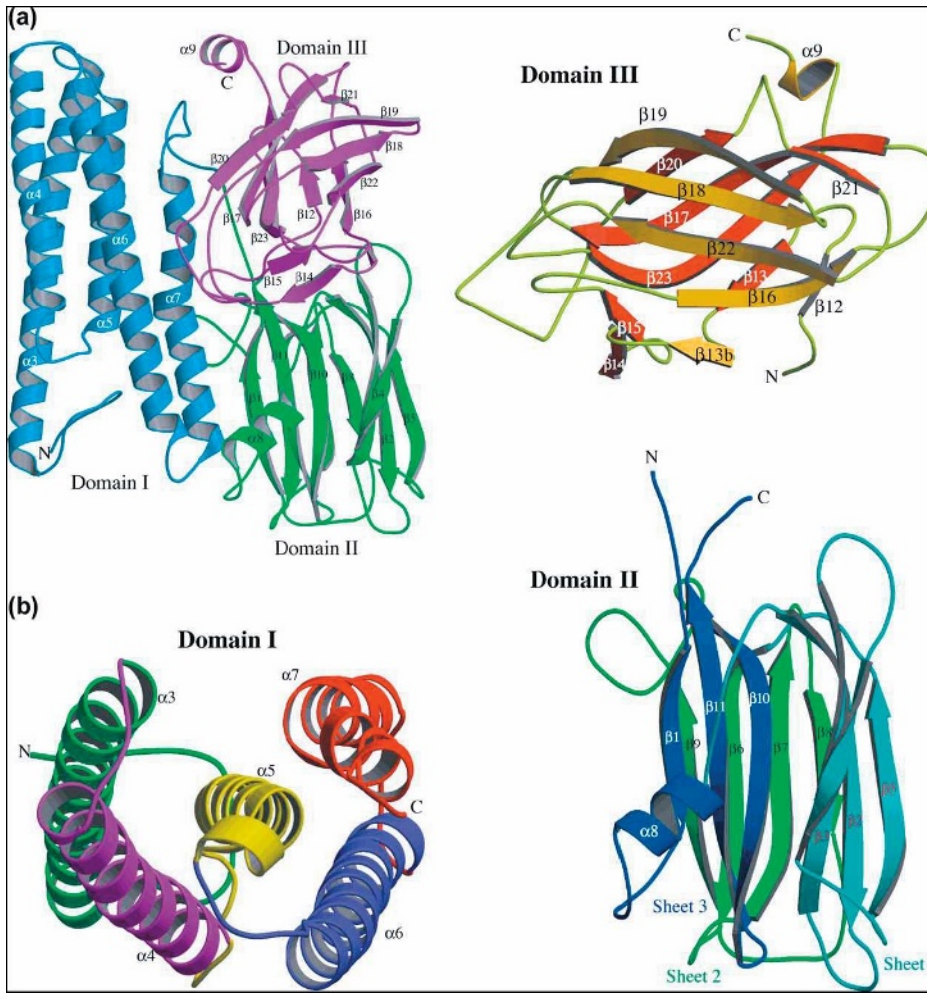

Fig. 2. Ribbon diagrams of the Cry4Ba toxin structure of *Bacillus thuringiensis* serovar. *israelensis*. (a) overview with domains I, II and III colored cyan, green and magenta, respectively; (b) the individual domains. From Booserm et al. (2005). Figure courtesy of David Ellar with permission from the Journal of Molecular Biology.

Fig. 3. Sporulating cell and parasporal body of *Bacillus sphaericus*. Figure courtesy of Jean-François Charles.

Alternative interpretations on the structure and mode of action of the Cyt1A toxin of *Bti* are presented in the literature. The first hypothesis on its mode of action is that the toxin assembles into defined cation-selective channels that result in osmotic lysis of the cell (Li et al. 1996, Promdonkoy and Ellar 2000, 2003). Alternative interpretations on the structure and mode of action of the Cyt1A toxin of *Bti* are presented in the literature. The alternative hypothesis is that Cyt1A non-specifically aggregates on the membrane surface and acts in a detergent-like manner (Butko et al. 1997, Butko 2003, Manceva et al. 2005). Pérez et al. (2005) provide evidence that Cyt1Aa functions as a receptor of Cry11Aa. Sequential-binding analysis of Cyt1Aa and Cry11Aa by these authors revealed that Cyt1Aa binding to *Aedes aegypti* brush border membrane vesicles enhanced the binding of biotinylated-Cry11Aa.

Although the modes of action of each of the Cry 4 and Cry 11A toxins are similar, they each attach to a distinct binding site on midgut epithelial cells. When the Cyt1A and Cry (Cry 4a, 4b, 11a) toxins are individually assayed, they cause mortality in *Ae. aegypti* larvae (Crickmore et al. 1995). However, bioassays of mixtures of the individual toxins revealed a number of synergistic interactions that explain in part why the native crystal is considerably more toxic than any of the individual toxins (Crickmore et al. 1995). Synergistic interactions between the Cyt1A and Cry toxins and synergistic interactions among the Cry proteins in *Cx. quinquefasciatus* have also been reported by Wirth and Georghiou (1997), Wirth et al. (1997, 2005b) and Pérez et al. (2005). The complex of toxins and separate sites of insertion are the key reasons why there has been little or no development of resistance despite long-term use in several control programs (see section on resistance).

Histopathology and Pathogenesis.

The midgut histopathology and pathogenesis of *Bti* toxins in mosquito larvae was studied by de Barjac (1978a), Charles and de Barjac (1981, 1983), Lahkim-Tsror et al. (1983), Mohsen et al. (1987), Yu (1989), Lee et al. (1991), and Rey et al. (1998). Within minutes of ingestion of a lethal dose of *Bti* toxins, there is noticeable swelling in the brush border of epithelial cells (blebbing). In most accounts, lytic vacuoles become apparent soon after and cells begin to lyse in as little as 30–60 min depending on dose of toxin. Eventually the affected cells are sloughed with only the basal membrane of the midgut remaining. Death of the insect follows soon thereafter. Lahkim-Tsror et al. (1983) reported that mortality in some larvae started as early as 6 min after application of high concentrations of *Bti*. The mode of action and histopathology of *Bti* toxins in mosquito larvae and that of other *Bt* strains in other insect targets are similar (Lüthy and Wolfersberger 2000).

Efficacy and Factors Affecting Activity of *Bti*

The efficacy of *Bti* formulations has been demonstrated in a variety of habits against a multitude of mosquito species (Table 1). Currently it is the most widely used microbial control agent of mosquitoes and black flies. Several biotic and abiotic factors influence the larvicidal activity of *Bti* including species of mosquito and their respective feeding strategies, rate of ingestion, age and density of larvae, dosage, habitat factors (temperature, solar radiation, depth of water, turbidity, tannin and organic content, presence of vegetation, etc.), formulation factors (type of carrier, toxin content, how effectively the material reaches the target, and settling rate), storage conditions, means of application and frequency of treatments, and production factors, especially the medium in which the bacterium is grown (Mulligan et al. 1980, Sinègre et al. 1981, Lacey and Undeen 1986, Wraight et al. 1987, Aly 1988, Aly et al. 1988, Kramer et al. 1988, Kramer 1990, Mulla et al. 1990b, Becker et al. 1992, Lord and Undeen 1990, Walker 1995, Beck et al. 1996, Skovmand et al. 1997, Boisvert and Boisvert 1999, Nayar et al. 1999, de Melo-Santos et al. 2001, Christiansen et al. 2004, Vilarinhos and Monnerat 2004). Although the bacterium is used for control of larvae, Stoops (2005) showed that females of *Aedes albopictus* laid more eggs in containers treated with *Bti* compared to untreated controls. This attract-and-kill phenomenon could improve efficacy especially when compared to larvicides that may be repellent to oviposition.

The persistence of *Bti* larvicidal activity is severely curtailed in organically enriched habitats where toxins are rapidly denatured and/or bound to organic matter (Mulligan et al. 1980, Hougard et al. 1983, Rathburn et al. 1984, Karch et al. 1991, Mulla et al. 1993, Srivastava et al. 1998, Russell et al. 2003). Rapid settling rate of *Bti* toxins can also shorten residual activity especially against mosquito species that feed at the surface (*Anopheles* spp.) or do not feed at deeper levels of the aquatic habitat (Standaert 1981, Hougard et al. 1983, Mullen and Hinkle 1988). Turbidity (suspended soil, sediments and organic matter) can also accelerate settling (van Essen and Hembree 1982, Margalit and Bobroglio 1984, Sheeran and Fisher 1992). Mulla et al. (1993) showed that elevated dosages of *Bti* failed to extend the duration of control of *Culex* species in organically enriched ponds. Under most conditions there is curtailed persistence of larvicidal activity requiring reapplication when mosquito breeding is continuous. However, extended control of *Ae. aegypti* in potable water reservoirs and other containers has been demonstrated with various formulations (Kroeger et al. 1995, Batra

Table 1. Selected examples of the use of *Bti* formulations for control of mosquito larvae in various habitats.

Formulation	Habitat	Principal targeted species	Selected references
Granules[a]	artificial pools	*Aedes vexans, Ochlerotatus trivittatus*	Clarke and Rowley 1984
	rice field	*Anopheles quadrimaculatus, An. crucians*	Lacey and Inman 1985
	experimental ponds	*Culex tarsalis, Cx. peus*	Mulla and Darwazeh 1985
	flooded pasture	*Psorophora columbiae*	Mulla et al. 1985
	tires	*Ae. aegypti*	Novak et al. 1985
	lagoons and pools	*Cx. pipiens*	Berry et al. 1987
	floodwater habitats	*Ae. vexans*	
	ditches ad culverts	*Cx. pipiens, Cx. restuans, Cx. tarsalis*	
	wild rice fields	*Cx. tarsalils, An. freeborni, An. franciscanus*	Kramer et al. 1988
	containers	*Oc. taeniorhynchus*	Ali et al. 1994
	mangrove swamp	*Oc. taenriorhynchus*	Lawler et al. 1999
	cesspits	*Cx. quiquefasciatus*	Skovmand and Sanago 1999
	rain puddles	*An. gambiae*	
	experimental tubs	*Cx. quinquefasciatus, Cx. stigmatosoma, Cx. tarsalis*	Su and Mulla 1999
	tires and coolers	*Ae. aegypti*	Batra et al. 2000
	rice plots	*An. quadrimaculatus*	Dennett and Meisch 2000
	tires	*Oc. triseriatus*	Siegel et al. 2001
	polluted pools	*Cx. annulirostris*	Russell et al. 2003
	tidal pools	*Oc. squamiger*	Christiansen et al. 2004
	containers	*Ae. aegypti*	Vilarinhos and Monnerat 2004
Ice granules (IcyPearls)	riparian woodland	*Ae. vexans*	Becker 2003
Wettable powder	peridomestic habitats	*An. stephensi*	Kumar et al. 1998
Flowable concentrate	experimental ponds	*Culex tarsalis, Cx. peus*	Mulla and Darwazeh 1985
	ditches and culverts	*Cx. pipiens*	Berry et al. 1987
	floodwater habitats	*Ae. vexans*	Gharib and Hilsenhoff 1988
	tires	*Culiseta incidens*	Kramer 1990
	field ponds	*Cx. quinquefasciatus, Cx. stigmatosoma, Cx. tarsalis*	Mulla et al. 1993
	woodland pools	*Oc. stimulans, Oc. intrudens, Oc. provpcans, Oc. canadensis*	Knepper et al. 1994
	tires	*Ae. albopictus*	Becnel et al. 1996
	cesspits	*Cx. quinquefasciatus*	Skovmand and Sanago 1999
	rain puddles	*An. gambiae*	
	artificial pools	*Cx. quinquefasciatus*	Hallmon et al. 2000
	polluted pools	*Cx. annulirostris*	Russel et al. 2003
Flowable concentrate (ULV)	rice field	*An. quadrimaculatus*	Yates 1984
	rice field	*An. quadrimaculatus*	Sandoski et al. 1985
	tires, containers	*Ae. aegypti, An. maculates, Cx. quinquefasciatus*	Lee et al. 1996
	containers	*Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus*	Yap et al. 1997
Oil + technical powder	experimental rice plots	*An. quadrimaculatus*	Dennett et al. 2000

Table 1. Continued.

Formulation	Habitat	Principal targeted species	Selected references
Floating tablets/pellets	containers	*Cx. quinquemaculatus*	Lacey et al. 1984
	woodland ponds	*Oc. cataphylla, Oc. cantans*	Shovman and Eriksen 1993
	containers	*Ae. albopictus*	Nasci et al. 1994
	Tires and evaporative coolers	*Ae. aegypti*	Batra et al. 2000
	water containers	*Ae. aegypti*	de Melo-Santos et al. 2001
	water containers	*Ae. albopictus*	Toma et al. 2003
	water containers	*Ae. aegypti*	Mulla et al. 2004
	containers	*Ae. aegypti*	Vilarinhos and Monnerat 2004
Briquettes	large containers	*Ae. aegypti*	Novak et al. 1985
	woodland pools	*Cx. territans, Cx. restuans*	Apperson et al. 1986
	farm pond	*Coquilletidia perturbans*	Walker 1987
	containers	*Ae. aegypti, Cx. pipiens, Cx. coronator*	Neri-Barbosa et al. 1997
	dambo ground pools	*Oc. macintoshi, Cx. antennatus*	Logan & Linthicum 1992
Floating bait	containers	*Anopheles spp.*	Aly et al. 1987
Microencapsulation	lab	*An. freeborni*	Cheung and Hammock 1985
Bioencapsulation	lab	*An. stephensi*	Manasherob et al. 1996

[a] Granules include water dispersible granules, corn cob grits, sand granules

et al. 2000, Mulla et al. 2004, Vilarinhos and Monerat 2004).

Germination, sporulation and recycling of *Bti* in larval cadavers in the laboratory and in simulated habitats have been reported by several authors (Aly et al. 1985, Khawaled et al. 1990, Pantuwatana and Sattabongkot 1990, Boisvert and Boisvert 1999), but this has not been observed under natural field conditions. Siegel et al. (2001) observed extended larvicidal activity against *Ochlerotatus triseriatus* in tires that had been treated 9 months earlier with *Bti*, but the authors could not determine if the mortality resulted from recycling of *Bti* or whether crystal proteins from the original treatment were resuspended.

Formulation and Application Factors

Formulation of *Bti* can help to overcome several of the factors that limit or reduce its larvicidal activity. Proper formulation can improve control performance by enabling greater contact with target larvae, ensuring stability under storage and field conditions, providing a variety of application options and increasing the ease of handling. The need to control mosquito larvae in a diversity of habitats has resulted in the production of a variety of formulations (Table 1). Granules, for example, enable penetration of habitats with dense foliage (rice fields, salt marsh, etc.), in tire piles, tree holes, and the like. Control of container-breeding mosquitoes, especially vector species such as *Ae. aegypti* and *Ae. albopictus* in domestic water storage vessels, requires slow release formulations that permit longer term control or high potency formulations that can be directly applied and have a minimum of effect on water flavor and appearance (Mulla et al. 2004, Vilarinhos and Monerat 2004). Aqueous suspension (AS) formulations, suspension concentrates (SC), flowable concentrates (FC) and aqueous suspensions of water dispersible granule (WDG) formulations can be applied using a variety of spray equipment including ultra low volume (ULV) generators. Surface feeding *Anopheles* larvae are best controlled by formulations that do not readily sink below the surface tension of the water. In addition to floating granules, aerially applied ULV flowable concentrates of *Bti* have provided excellent control of *Anopheles* species (Yates 1984, Sandoski et al. 1985) in rice fields. The small droplets (volume median diameter $\approx$80 μm) produced with the Beecomist® ULV generator penetrate dense foliage and remain in the feeding zone of *An. quadrimaculatus* larvae longer than larger droplets or granules that sink. Ground-applied ULV *Bti* for control of container-breeding mosquitoes was demonstrated by Lee et al. (1996) and Yap et al. (1997) as an effective method for penetrating breeding sites (tires, water

containers) for control of *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus*, and *An. maculatus*.

Microencapsulation may help to ameliorate some of the problems of short residual activity due to microbial and solar degradation of toxin (Margalit et al. 1984, Cheung and Hammock 1985, Vorgetts and Buescher 1985, Lee et al. 2003). Cheung and Hammock (1985) microencapsulated purified *Bti* toxin in lipid droplets that floated on the surface and improved larvicidal activity for *An. freeborni*. The formulation of solubilized toxin has received limited attention. Lee et al. (2003) demonstrated that larvicidal activity of solubilized *Bti* toxins that were bound to various types of clay persisted in non-sterile water after 45 days and was significantly greater than that of unbound free *Bti* toxins.

One of the more unique formulations of *Bti* and *B. sphaericus* was produced by Becker (2003) in frozen granules named "IcyPearls." The formulation was demonstrated to have certain advantages over *Bti* sand granules: (1) ice pellets melted on the water surface and released the microbial toxins there; (2) the control agent remained inside the ice pellets during the application and was not lost by friction in the spraying equipment; and (3) the ice formulation resulted in increased swath widths, thereby reducing the cost of application. In large field tests, applications of "IcyPearls" against *Ae. vexans* larvae in the Rhine River Valley of Germany resulted in mortality rates of 91–98%. Another unique "formulation" is the concentration of *Bti* in the protozoan *Tetrahymena pyriformis* (Manasherob et al. 1996, Ganushkina et al. 1997). Interestingly, Manasherob et al. (1998) also reported germination, growth, and sporulation of *Bti* in excreted food vacuoles of *T. pyriformis*.

In certain situations the use of live-spore formulations of *Bti* may be prohibited (i.e., treatment of drinking water). Lacey and Smittle (1985) studied the effect of gamma radiation on spore viability and insecticidal activity of *Bti* using a cobalt-60 source irradiator. Gamma radiation from 50–1750 kiloroentgens (Kr) progressively lowered viability of a *Bti* standard reference powder (IPS-78). Significant loss of insecticidal activity was observed only with radiation over 1000 Kr. At 1500 Kr there was a 99.9999% reduction in spore count with a corresponding 17% loss of insecticidal activity. In habitats treated with irradiated *Bti* products, Becker (2002) reported that either no spores or fewer than 10^5 spores per gram of soil were found compared with 7.0×10^5 to 4.4×10^6 spores per gram of soil in habitats treated with nonirradiated *Bti* products twice (on average) per year.

Storage Conditions

Storage conditions and duration of storage can also affect the activity of *Bti*. When properly stored (at room temperature or cooler) larvicidal activity can remain unaffected for several months or even years (Sokolova et al. 1987, Thiery and Hamon 1998). Thiery and Hamon (1998) bioassayed the IPS-82 *Bti* standard against *Ae. aegypti* and considered it stable over a 16-year evaluation period. The negative effect of freezing and thawing on larvicidal activity of stored *Bti* is reported by Tousignant et al. (1992). The number of freeze-thaw cycles was correlated with an increase in LC_{50} values. Boisvert and Boisvert (2001) studied the effect of storage at room temperature and freezing over time on 2 liquid commercial formulations of *Bti* (Vectobac 1200L and Teknar HP-D) and reported that the LC_{50} values when bioassayed against *Oc. triseriatus* for both increased by a factor of 2.5 after 6 months of freezing. At room temperature the Teknar HP-D formulation was stable for 2 years with an increase in LC_{50} of 20% during the 3rd year. Vectobac 1200L was stable during the 1st year, but LC_{50} values increased by 22 and 20% during the 2nd and 3rd years of storage, respectively. Karch (1989) reported good stability of larvicidal activity of the IPS-82 spore powder standard that was stored at 50°C for 9 months. The effect of storage temperature and duration on larvicidal activity of *Bti* formulations is also presented by Balaraman and Hoti (1984), Sokolova et al. (1987) and Farghal and Darwish (1988).

Fermentation Factors

The nutrient media used and conditions under which *Bti* and other serovarieties of *Bt* are produced can markedly influence larvicidal activity. Dulmage et al. (1990) and Beegle et al. (1991) provide guidelines for small- and large-scale production. Beegle et al. (1991) reviewed key aspects of *Bt* large-scale production using semi-solid and submerged fermentation. The basic nutritional requirements of *Bt* are sources of carbon, nitrogen, and trace minerals. Several examples of media used for shake flask and stir tank production are provided by Dulmage et al. (1990) and Beegle et al. (1991). The T-3 medium [tryptose (2 g/liter), tryptone (3 g/liter), yeast extract (1.5 g/liter), and trace minerals ($MnSO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$, $NaH_2PO_4 \cdot H_2O$), adjusted to pH 6.8], for example, is a light soluble medium that has been useful for growing a wide variety of *Bt* isolates in shake flasks. Heavier media are used for commercial production and contain ingredients such as soybean and cottonseed flour. The disadvantages of these heavier media are the non-utilized insolubles in the flours that remain with the spore-inclusion complexes when they are recovered. Rossa and Mignone (1993) studied spore production in batch and

fed-batch cultures of *Bti*. They found that spore counts reached in both cultures were comparable, but the levels of toxicity of the fed-batch cultures were about one order of magnitude lower than that of the batch culture. The use of local raw ingredients and bi-products for the production of *Bti* is presented by Chilcott and Pillai (1985), Dharmsthiti et al. (1985), Yoon et al. (1987), Morsi and Abdel-Samie (1988), Ejiofor and Okafor (1989), Kuppusamy and Balaraman (1991), Subbiah and Kumar (2003), Subbiah et al. (2003), and Prabakaran and Balaraman (2006). Dulmage et al. (1990) list several examples of inexpensive media components of plant and animal origin that could be available as carbohydrate and protein sources for production of *Bti* in developing countries.

Field Evaluation Factors

Experimental design and the manner in which *Bti* formulations are evaluated can influence the interpretation of results. Individual protocols for field evaluation of *Bti* differ considerably from one habitat and mosquito species to another. Skovmand et al. (2000a) recommend that the selection of test plots should consider at least 3 aspects: (1) whether the site is representative of the target species; (2) different environmental conditions for the target insect in the area to be treated (monitoring of environmental conditions such as temperature, water quality and chemistry, solar exposure, etc., will enable more quantitative comparison between sites); (3) compatibility of the test site and *Bti* formulation. The number and distribution of sites will depend on several factors including purpose of the study, available resources for treatment and sampling, larval distribution and density, habitat, and life cycle of the target mosquito. Specific examples for the field evaluation of microbial mosquito larvicides in container habitats, temporary pools, and large permanent or semi-permanent habitats are presented by Skovmand et al. (2000a).

Resistance

Resistance in Lepidoptera and other invertebrate systems to Cry toxins has been associated with deficient protoxin activation by host proteases and defective Cry toxin binding to cell surface molecules (Crickmore 2005, Griffitts and Aroian 2005). Recent evidence also suggests that Cry toxin resistance may be induced in invertebrates as an active immune response (Griffitts and Aroian 2005). Although elevated levels of resistance to *B. thuringiensis* serovar. *kurstaki* and other serovarieties have been reported in lepidopteran species (van Rie and Ferré 2000), repeated challenges of mosquito species with the full complement of *Bti* toxins in controlled laboratory studies have failed to develop significant resistance (Goldman et al. 1986, Georghiou and Wirth 1997). However, when the number of toxins produced by *Bti* has been limited to less than the natural complement of 4 toxins, especially when populations are repeatedly challenged with single toxins, significant resistance has been induced (Georghiou and Wirth 1997, Wirth and Georghiou 1997, Wirth et al. 2003). Repeated challenges of larvae with combinations of *Bti* Cry 4 toxins in the absence of Cyt A toxins have also produced resistance (Wirth et al. 1997, 2003). Wirth et al. (1997, 2005b) demonstrated that the Cyt A enables Cry 4 and Cry11 A endotoxins to overcome or delay development of resistance in *Cx. quinquefasciatus*. Data provided by Pérez et al. (2005) indicate that Cyt1Aa synergizes or suppresses resistance to Cry11Aa toxin by functioning as a membrane-bound receptor. Mosquitoes with documented resistance to *B. sphaericus* toxins have not been demonstrated to be cross-resistant to *Bti* (Rodcharoen and Mulla 1996, Wirth et al. 2000b, Yuan et al. 2003).

The vast majority of field populations of mosquitoes that have received repeated applications of *Bti* over several years have not developed resistance to the bacterium (Becker and Ludwig, 1993; Regis and Nielsen-LeRoux, 2000). However, Paul et al. (2005) recently reported high levels of resistance to *Bti* in an isolated field population of *Cx. pipiens* in Syracuse, NY. Further study of this population, the mechanism responsible for resistance and its management are highly warranted.

Safety and Ecotoxicology

With the exception of certain species of nontarget Nematocera, *Bti* is safe for other nontarget organisms including the vast majority of insects, other invertebrates and vertebrates. The specificity of *Bti* has been documented in a multitude of studies from 1978 to the present. The extensive literature has been summarized and reviewed by Dejoux and Elouard (1990), Lacey and Mulla (1990), Saik et al. (1990), Siegel and Shadduck (1990b, 1990c), Boisvert and Boisvert (2000), Glare and O'Callaghan (2000), Lacey and Siegel (2000), and Lacey and Merritt (2003). Studies on the effects of *Bti* on invertebrate nontarget organisms include those of Aly and Mulla (1987), Gharib and Hilsenhoff (1988), Kramer et al. (1988), Merritt et al. (1989), Molloy (1992), Charbonneau et al. (1994), Wipfli and Merritt (1994b), Hershey et al. (1995, 1998), Neri-Barbosa et al. (1997), Becker (1998), and Lawler et al. (1999, 2000). The toxins that are responsible for the pathogenic effect in mosquito larvae have no effect on vertebrates including fish, birds, and mammals (Fortin et al. 1986, Lee and Scott 1989, Merritt et al. 1989, Saik et al. 1990, Siegel and Shadduck 1990b, 1990c; Wipfli et al. 1994, Siegel 1997). However, when the solubilized Cyt1A toxin of *Bti* is injected into cell cultures, it has

a cytolytic effect on the cells. The amount of this toxin and the way in which it is applied for mosquito control poses virtually no threat to non-target organisms including birds and mammals. Guidelines for testing the pathogenicity and infectivity of *Bti* and other entomopathogens to mammals are presented by Siegel (1997).

Several studies on the effects of *Bti* formulations on fish are reviewed by Lacey and Siegel (2000) and Lacey and Merritt (2003). None of the formulations tested produced deleterious effects to fish when label rates were used. Wipfli et al. (1994) exposed the early stages of 3 species of trout to extremely high concentrations of *Bti*, but mortality was only observed at a concentration 70 times greater and an exposure 192 times longer than that recommended for black fly control. Mortality of fish was attributed to formulation components and not to *Bti* toxins. Fortin et al. (1986) reported similar observations in brook trout fry that were exposed to extremely high concentrations of a liquid formulation of *Bti*, but concluded that mortality was due to formulation components.

In addition to mosquito larvae, some *Bti*-susceptible non-target species are found in several families of Nematocera including Simuliidae (pest species), Chironomidae (pest and non-target), Tipulidae (pest and non-target), Psychodidae (pest and non-target), Chaoboridae (pest and non-target), Sciaridae (pest) and Blepharoceridae (non-target) (Boisvert and Boisvert 2000, Lacey and Merritt 2003). Larvae of predatory beneficial mosquitoes in the genus *Toxorhynchites* are susceptible to *Bti* if they feed upon larvae of prey species, such as *Ae. aegypti* that have fed on suspensions of the bacterium (Lacey and Dame 1982, Larget and Charles 1982, Lacey 1983). Several laboratory and field studies have demonstrated larvicidal activity for certain species of chironomid midges (Chironomidae) including pest species (Ali 1981, Merritt et al. 1989, Mulla et al. 1990a, Rodcharoen et al. 1991) and non-target species (Molloy 1992, Hershey et al. 1998, Liber et al. 1998, Pont et al. 1999, Yiallouros et al. 1999, Hughes et al. 2005).

Few studies on the long-term effects of *Bti* have addressed more than direct toxicity of the bacterium for nontarget organisms. The ecotoxicological effects are much more complex, involving effects of removal of a major prey species on aquatic community diversity and structure. Mosquitoes and other targeted Nematocera often contribute substantial biomass to aquatic food webs. If their populations are severely suppressed on a continuous basis and their role in the ecosystem is significant, it could result in reductions of specialized predator species that depend upon them as a major food source. The more diversified the food web, the less likely that complete or partial removal of a single species will result in catastrophic consequences. Becker (1998) observed no long-term deleterious effect of *Bti* on non-target organisms that are monitored as part of the Rhine River mosquito control program. In contrast, long-term monitoring of a wetland ecosystem in Minnesota indicated that multiple, routine treatments at relatively high application rates may impact certain benthic non-target organisms. Five applications of a 200 ITU *Bti* formulation were made at 8.5 lb/acre/application during the 1st year, and 6 applications were made at 10 lb/acre/application during the 2nd year. No short-term changes of the ecosystem were observed in these years, but during the 3rd year, when wetlands were treated 6 times at 13.5 lb/acre/application with the formulation, species diversity and richness declined significantly (Hershey et al. 1995, 1998). Primary taxa reduced in this study were immature mosquitoes and chironomids. Because applications were made routinely, and were not based on monitoring of larval mosquito populations (as is the normal operational practice in mosquito control), more applications were made than would be expected operationally. Rates applied during each treatment in years 2 and 3 were also higher than the operational rate of 5–8 lb/acre used for floodwater mosquito control in Minnesota (Anderson 1996). It is not clear whether effects on chironomids in year 3 in the studies of Hershey et al. (1995, 1998) were cumulative or dose related, because the total amount of the formulation applied each year increased from 42.5 lb/acre in year 1 to 80.9 lb/acre in year 3. Investigations by Schmude et al. (1997) and Balcer et al. (1999) in the same habitat did not corroborate the findings of Hershey et al. (1998) and revealed no long-term effect on insect community structure due to repeated *Bti* applications. Investigations by Hanowski et al. (1997) in these wetlands showed no long-term effect on bird diversity and numbers due to repeated *Bti* applications.

Molloy (1992) observed very little effect on most non-target organisms after multiple applications of *Bti* in small streams for black fly control in New York State. Merritt et al. (1991) and Wipfli and Merritt (1994a, 1994b) observed changes in feeding habits of selected nontarget aquatic insects and disturbance to a stream food web due to use of *Bti* for black fly control. They concluded that specialist predators in black fly-poor environments would be most affected, whereas generalist predators would be least affected. In order to predict potential detrimental effects of routine use of *Bti* in aquatic ecosystems, a better understanding of the basic ecological interactions within the food web between pathogen and target and non-target organisms is warranted.

BACILLUS SPHAERICUS

GENERAL BACKGROUND

Bacillus sphaericus is a gram-positive, spore forming, aerobic bacterium found in a multitude of soil and aquatic habitats. The species *B. sphaericus* comprises 5 major homology groups that share only a few characteristics (spherical terminal spore, inability to ferment sugars) (Berry et al. 1991, Charles et al. 1996). Only the IIA subgroup has isolates with insecticidal activity. Singer (1985) presented the research and initial commercial development of *B. sphaericus* prior to 1985. Much of the earlier work was conducted with the SSII-1 isolate and its 100 kDa toxin. This toxin is produced by vegetative cells and is present in both highly and weakly insecticidal strains of *B. sphaericus* (Davidson 1995). Since the discovery of the highly mosquitocidal 1593 isolate in Indonesia, several other isolates have been found with excellent larvicidal activity in mosquitoes. The serovarieties of *B. sphaericus* with the most pronounced larvicidal activity are 5a5b (isolates 1593, 2013, 2362) and 25 (isolate 2297). The majority of the published research has been conducted on the 1593 and 2362 isolates. Most commercial formulations of *B. sphaericus* are based on the 2362 isolate. Abbott Laboratories developed the first *B. sphaericus* products in North America that are now formulated and marketed by Valent BioSciences Corporation, USA.

Protocols for the bioassay of larvicidal activity of *B. sphaericus* primary powders and formulations are presented by Lacey (1997) and Thiery et al. (1997). The potency of commercial products is usually based on comparison with standard reference powders. Several of the standards have been produced by the Pasteur Institute and are listed by Bourgouin et al. (1984) and Thiery and Hamon (1998).

Toxins and Mode of Action

The moiety responsible for mosquito larvicidal activity in serovar. 5a5b isolates of *B. sphaericus* is a binary toxin (Charles et al. 1996). As with *Bti*, ingested toxins are solubilized in the alkaline midgut and cleaved to the active moiety by proteases. The 2 component proteins of the toxin, BinA (42 kDa) and BinB (51 kDa) bind to specific receptors on the brush border of epithelial cells of the gastric caecum and midgut and cause pore formation (permeabilization) resulting in disruption of osmotic balance, lysis of the cells, and ultimately death of the insect (Davidson 1988, Davidson et al. 1990, Nielsen-LeRoux and Charles 1992, Oei et al. 1992, Charles et al. 1997, Silva-Filha et al. 1997, 1999; Darboux et al. 2001, Schwartz et al. 2001). Both proteins are required for full toxicity (Davidson et al. 1990). The individual roles of the toxin components have been elucidated by Charles et al. (1997) and Schwartz et al. (2001). In experiments with non-susceptible *Ae. aegypti* larvae, Nielsen-LeRoux and Charles (1992) did not detect significant binding of the binary toxin to the brush border membrane. Humphreys and Berry (1998) report variants of the binary toxins and discuss the implications for differential toxicity of strains.

Although the binary toxin is located in an inclusion that is formed during sporulation and causes a similar pathology to that of the Cry and Cyt toxins of *Bti* and other *Bt* strains, it is not related to these toxins. However, the binary toxin of the *Bt* serovar. *morrisoni* HnC isolate does share certain similarities with the 2 *B. sphaericus* toxin components, but does not share sequence homology (R. Frutos, unpublished data).

Three other toxins (Mtx1, Mtx2, Mtx3) are produced in low quantities by vegetative *B. sphaericus* cells. Although they have a toxic effect on the midgut cells of *Cx. quinquefasciatus*, their contribution to the insecticidal activity of formulations used in control programs is negligible. Myers et al. (1979) compared the activity of toxin produced by the SSII-1 strain (i.e., Mtx 1) with that of the 1593 binary toxin and found the latter to be 3000 times more toxic to *Cx. quinquefasciatus*. Laboratory assays against several culicine species by Wraight et al. (1987) determined that the potency of the SSII-1 toxin they used was roughly one-tenth that of the 1593 and 2013-4 isolates.

Histopathology and Pathogenesis

The cytopathology and pathogenesis of *B. sphaericus* binary toxin in the midgut of *Culex* larvae and cultured mosquito cells was studied by Menon et al. (1982), Karch and Coz (1983), Charles (1987), Davidson and Titus (1987), Mohsen et al. (1987), and Singh and Gill (1988). The histopathology is similar to that observed with *Bti* in that midgut osmotic balance is disrupted and numerous cytolytic vacuoles form in midgut epithelial cells followed by lysis of the cells (Davidson 1981). Epithelial cells in the gastric caeca and posterior midgut are the cells most severely damaged by the binary toxin. In as little as 15 min. after ingestion of concentrated binary toxin there is noticeable swelling in the brush boarder of epithelial cells (blebbing), mitochondrial swelling and cytolytic vacuoles become apparent soon afterward. Davidson et al. (1975) and Davidson (1979) reported on the pathogenesis and cytopathology due to the toxin produced by the SSII-1 strain of *B. sphaericus*.

Efficacy and Factors Affecting Activity of *B. sphaericus*

Excellent control of larvae of certain mosquito species, especially in the genera *Culex* and *Psorophora* and, to a lesser extent *Anopheles, Aedes, Ochlerotatus* and *Mansonia* species, has

been reported for *B. sphaericus* (Table 2). *Stegomyia (Aedes)* species are largely unaffected by *B. sphaericus. Bacillus sphaericus* is affected by many of the same biotic and environmental factors as *Bti* including species of mosquito, feeding strategy, rate of ingestion, age and density of larvae, temperature, solar radiation, depth of water, turbidity and organic content, type of formulation, storage conditions, fermentation factors, means of application and frequency of treatments (Mulligan et al. 1980, Davidson 1981, 1984; Lacey 1984, Burke et al. 1983, Lacey and Undeen 1986, Wraight et al. 1987, Lacey et al. 1988b, Mulla et al. 1990b, Kramer 1990, Yousten and Russell 1990, Ludwig et al. 1994, Skovmand and Bauduin 1997).

Bacillus sphaericus binary toxin is more specific than the *Bti* toxins, being principally active against mosquitoes. The range of mosquito species that are affected by *B. sphaericus* is also narrower than that of *Bti*. For example, the effect of *B. sphaericus* toxins on *Ae. aegypti* larvae is low to negligible for most isolates (Davidson 1981, Wraight et al. 1987, Lacey et al. 1988b, Thiery and de Barjac 1989, Berry et al. 1993, Davidson 1988, 1995; Monnerat et al. 2004). On the other hand, several *Ochlerotatus* (=*Aedes*) species are moderately susceptible to the bacterium (Lacey and Singer 1982, Wraight et al. 1987, Lacey et al. 1988b, Mulla et al. 1988b, Siegel et al. 1996, 2001). The bacterial genetic determinants of the host ranges of *B. sphaericus* mosquito larvicidal toxins was reviewed by Berry et al. (1993). Minor variations in the toxicity among strains of 5a5b serovarieties are likely due to the presence of other toxins in addition to the binary toxin (Berry et al. 1993). Wirth et al. (2000a, 2001, 2004) demonstrated that the Cyt toxins from *Bti* and *Bt* serovar. *medellin* synergize the larvicidal activity of *B. sphaericus* for *Ae. aegypti*. A ratio of 10:1 of *B. sphaericus* to Cyt 1A was 3600 times more toxic to *Ae. aegypti* larvae than *B. sphaericus* alone (Wirth et al. 2000a).

Formulation

Despite its narrower host range, *B. sphaericus* formulations are as diversified as that of *Bti*. A variety of granules, flowable concentrates, water dispersible granules, and slow release formulations in addition to some more novel formulations are available for use with a variety of conventional equipment. The combination of formulation and application strategy enables treatment of virtually any mosquito habitat (Table 2). The advantages of the various formulations and means of applying them are covered in the section on *Bti* formulations.

Persistence and Recycling

Bacillus sphaericus has the advantage of providing better residual activity in organically enriched habitats than *Bti*. Larvicidal activity and spores persist for prolonged periods of time in mosquito habitats (Mulligan et al. 1980, Silapanuntakul et al. 1983, Davidson et al. 1984, Des Rochers and Garcia 1984, Mulla et al. 1984a, Hornby et al. 1984, Nicolas et al. 1987, Karch et al. 1988, Arredondo-Jimenez et al. 1990, Hougard 1990, Kramer 1990, Lacey 1990, Matanmi et al. 1990, Siegel et al. 2001), and evidence for recycling has been reported in several studies (Davidson et al. 1975, Hertlein et al. 1979, Menon et al. 1982, Des Rochers and Garcia 1984, Karch and Coz 1986, Nicolas et al. 1987, Karch et al. 1988, Kramer 1990, Yuan et al. 1999). Karch et al. (1988) reported the presence of *B. sphaericus* spores in dead *Cx. pipiens* larvae and sediments in one site treated 4 years earlier. Although early instars were continually present, they rarely completed development.

Recycling of *B. sphaericus* in intact cadavers of *Cx. quinquefasciatus, Cx. pipiens* and other species may play a principal role in the maintenance of larvicidal levels of toxin in mosquito habitats (Davidson et al. 1975, Menon et al. 1982, Karch and Coz 1984, 1986; Nicolas et al. 1987, Kramer 1990, Pantuwatana and Sattabongkot 1990, Becker et al. 1995, Correa and Yousten 1995, Yuan et al. 1999). Apparently the intact cadavers contain the nutrients and conditions necessary for vegetative growth and sporulation of *B. sphaericus*. Crushed cadavers alone did not provide the conditions that facilitated growth and sporulation of the bacterium (Becker et al. 1995). Correa and Yousten (1995) showed that the rate of spore germination and recycling in larval cadavers varied among *B. sphaericus* strains. Strains producing the spore associated binary toxin had a high percentage of spore germination in cadavers whereas a strain that produces the vegetative cell associated toxin (Mtx) germinated less well. Insects other than mosquitoes and other arthropods were shown to contribute to recycling and dissemination of *B. sphaericus* in larval habitats (Karch et al. 1989, 1990; Yousten et al. 1991, 1992).

Despite the reported recycling and persistence of *B. sphaericus* larvicidal activity in several polluted habitats, settling of spores into substrates that are inaccessible to larvae can contribute to reduced longevity of larvicidal activity (Davidson et al. 1984, Lacey et al. 1988b, Mulla et al. 1997, Skovmand and Guillet 2000). Lacey et al. (1988b) observed curtailed activity of the 2297 isolate in a habitat that was highly organically enriched and deeper than the feeding range of the targeted *Cx. nigripalpus* larvae.

Although *B. sphaericus* spores are sensitive to direct UV light, Burke et al. (1983) considered the impact of solar radiation in aquatic habitats to be considerably reduced due to filtration of harmful rays by environmental components (water, algae and other seston). Despite the reduction of spore

viability after exposure to unfiltered UV light, insecticidal activity was quite resistant to inactivation (Burke et al. 1983). Similarly, Lacey and Smittle (1985) reported *B. sphaericus* spores that had been almost totally inactivated by gamma radiation (1750 Kr) still retained larvicidal activity.

In addition to larvicidal activity, the sublethal effects of *B. sphaericus* on surviving larvae may reduce the longevity and vector capacity and competence of mosquitoes. Lacey et al. (1987) observed prolongation of larval and pupal development, decreased emergence, and reduced survival and energy stores in adult *Cx. quinquefasciatus* that had survived exposure as larvae to an LC_{60} of the 2362 isolate.

Storage Conditions

The effect of storage conditions on spore viability and larvicidal activity has been investigated by several researchers. Lacey (1985b) studied the effect of storage temperature (4 and 21°C) and pH (3.0–10.8). Spore viability and larvicidal activity of buffered suspensions of *B. sphaericus* spores remained relatively high for as long as 3 months in pH 3–10 at 4°C. Those held at 4 and 21°C in pH 10.8 lost larvicidal activity within the 1st week, but spore counts remained high. For suspensions held at 4°C for 308 days, the larvicidal activity remained high for spores stored at pH 3–8 and spore counts remained high for suspensions in pH 5–10.8. Loss of spore viability and larvicidal activity at 21°C was accelerated considerably over that of the suspensions held at 4°C, with only negligible larvicidal activity remaining after 240 days. The best storage regime for spore viability and larvicidal activity was 4°C in neutral buffer.

Thiery and Hamon (1998) bioassayed the SPH88 *B. sphaericus* standard against *Cx. pipiens* and considered it stable over a 10-year evaluation period. Their study also demonstrated that the SPH88 standard could be kept in stock suspension at 4°C for 3 years without loss of potency. After 9 years of storage in suspension, only a 2-fold decrease in potency was observed. Balaraman and Hoti (1984) report on the rapid deterioration of water dispersible- granules of indigenous strains of *B. sphaericus* stored at −40, 8 and 30°C. Alves et al. (2001) detected better stability in *B. sphaericus* corn cob formulations over a 12-month period at 25–50°C, than in a concentrated spore-inclusion suspension stored at the same temperatures. High larvicidal activity of the corn cob formulation against *Cx. quinquefasciatus* was observed after 60 days at 50°C, whereas the suspension had lost all activity. Karch (1989) reported better viability for the 1593-4 strain (RB 80) than for 2297 (SPH 84) when spore powders were stored at 50°C for 9 months.

Fermentation Conditions and Media

Like *Bti*, the medium utilized and conditions under which *B. sphaericus* is produced can influence larvicidal activity (Dulmage et al. 1990, Yousten et al. 1990, Beegle et al. 1991). Dulmage et al. (1990) and Beegle et al. (1991) provide guidelines for small- and large-scale production. Lacey (1984) and Beegle et al. (1991) reviewed key aspects of small- and large-scale *B. sphaericus* production using semisolid and submerged fermentation. Unlike *Bt*, *B. sphaericus* does not utilize sugars as a carbon source and does not grow well with starch and several other carbohydrates. The medium used by Yousten and Wallis (1987), for example, contains beef extract, peptone, yeast extract and trace minerals ($MnCl_2 \cdot 4H_20$, $CaCl_2 \cdot 2H_20$, $MgCl_2 \cdot 6H_20$). Kalfon et al. (1983) describe the MBS medium for optimal sporulation of *B. sphaericus*. The use of locally acquired raw ingredients and bi-products for the production of *B. sphaericus* is presented by Hertlein et al. (1981), Dharmsthiti et al. (1985), Ejiofor and Okafor (1988), Bhumiratana (1990), Kuppusamy and Balaraman (1991), and Subbiah et al. (2003). Dulmage et al. (1990) list several examples of inexpensive media components that could be available for production of *B. sphaericus* in developing countries.

Field Evaluation

As discussed in the *Bti* section, experimental design and the manner in which *B. sphaericus* formulations are evaluated can influence the interpretation of results. Protocols for the short-term evaluation of *B. sphaericus* formulations in the field will, for the most part, be similar to that of *Bti* evaluations (Skovmand et al. 2000a). The duration of sampling could be considerably longer depending on environmental conditions. One major difference in assessments of efficacy of *B. sphaericus* relative to *Bti* is that sampling results should be segregated by instar in order to allow detection of delayed mortality. It is characteristic of some efficacy studies with *B. sphaericus* that recovery of 1st and 2nd instars, but not 3rd and 4th instars or pupae is observed during long term sampling. Methods for enumerating the number of spores in treated habitats using a selective medium are presented by Davidson et al. (1984). Individual protocols for field evaluation will differ considerably from one habitat and mosquito species to another. Thiery et al. (1997) discuss the complexity of standardizing outdoor protocols.

Resistance

Low to extremely high levels of resistance to the *B. sphaericus* binary toxin have been reported in populations of *Cx. quinquefasciatus* in India (Rao et al. 1995), Brazil (Silva-Filha et al. 1995,

Table 2. Selected examples of the successful use of experimental and commercial formulations of *Bacillus sphaericus* for control of mosquito larvae in variety of habitats.

Formulation	Habitat	Principal targeted species	Selected references
Granules	dairy wastewater	*Culex quinquefasciatus*	Mulla et al. 1984a
	artificial pools	*Cx. pipiens*	Berry et al. 1987
	wastewater lagoons	*Cx. pipiens, Ochlerotatus trivittatus*	
	rice field	*Psorophora columbiae, Anopheles quadrimaculatus, An. crucians*	Lacey et al. 1988c
	sod-lined potholes	*Cx. quinquefasciatus*	
	dairy wastewater	*Cx. peus*	Mulla et al. 1988a
	irrigated pasture	*Oc. nigromaculis*	Mulla et al. 1988b
	experimental potholes	*An. albimanus, Culex* spp.	Arredondo-Jimenez et al. 1990
	experiemntal rice field plots	*Ps. columbiae*	Bowles et al. 1990
	irrigation ponds	*An. gambiae*	Karch et al. 1991
	polluted gutter water	*Cx. quinquefacsciatus*	Karch et al. 1991
	rice paddy ditches	*Mansonia indiana, Ma. uniformis*	Yap et al. 1991
	tires	*Oc. triseriatus*	Siegel et al. 1999
	polluted ponds	*Cx. quinquefasciatus*	Mulla et al. 1997, 1999
	tires	*Oc. triseriatus, Cx. restuans, Cx. pipiens*	Siegel and Novak 1997
	containers, cess pools	*Cx. quinquesfasciatus*	Skovmand and Bauduin 1997
	sunlit ponds	*An. gambiae*	
	catch basins	*Cx. restuans, Cx. pipiens*	Siegel and Novak 1999
	tires	*Oc. triseriatus*	
	cesspits	*Cx. quinquefasciatus*	Skovmand and Sanogo 1999
	rain puddles	*An. gambiae*	
	experimental tubs	*Cx. quinquesfasciatus, Cx. stigmatosoma, Cx. tarsalis*	Su and Mulla 1999
	experimental rice field plots	*An. quadrimaculatus*	Dennet and Meisch 2000
	experimental rice field plots	*An. quadrimaculatus, Ps. columbiae*	Dennett et al. 2001
	various urban habitats	*Cx. quinquefasciatus*	Mulla et al. 2001
	tires	*Oc. triseriatus*	Siegel et al. 2001
	polluted pools	*Cx. quinquefasciatus, Cx. annulirostris*	Brown et al. 2004
Ice granules (IcyPearls)	riparian woodland	*Ae. vexans*	Becker 2003
Wettable powder	experimental rice field plots	*Ps. columbiae*	Bowles et al. 1990
	various polluted sites	*Cx. quinquefasciatus*	Kumar et al. 1996

Table 2. Continued.

Formulation	Habitat	Principal targeted species	Selected references
Flowable concentrate/ Aqueous suspension	rice field	*An. quadimaculatus*	Lacey et al. 1986
	reflooded rice field	*Ps. columbiae*	
	cesspools	*Cx. quinquefasciatus*	Nicolas et al. 1987
	wastewater	*Cx. nigripalpus*	Lacey et al. 1988b
	sod-lined potholes	*Cx. quinquefasciatus*	Lacey et al. 1988c
	dairy wastewater	*Cx. peus*	Mulla et al. 1988a
	irrigated fields	*Ps. columbiae, Oc. nigromaculis*	Mulla et al. 1988b
	experimental potholes	*An. albimanus, Culex* spp.	Arredondo-Jimenez et al. 1990
	experimental rice field plots	*Ps. columbiae*	Bowles et al. 1990
	cesspits	*Cx. quinquefasciatus*	Hougard 1990
	dairy effluent ditches	*Cx. quinquefasciatus*	Jones et al. 1990
	settling basin	*Cx. pipiens*	Karch et el. 1990
	tires	*Culiseta incidens*	Kramer 1990
	dairy wastewater	*Cx. stigmatosoma*	Matanmi et al. 1990
	polluted pools	*Cx. quinquefasciatus, An. funestus*	Ragoonanansingh et al. 1992
	various urban habitats	*Cx. quinquefasciatus*	Hougard et al. 1993
	rice field plots	*Ps. columbiae*	Groves and Meisch 1996
	various urban habitats	*Cx. quinquefasciatus, An. albimanus*	Barbazan et al. 1997, 1998
	lake shore habitats	*An. albimanus*	Rivera et al. 1997
	containers, cess pools	*Cx. quinquefasciatus*	Skovmand and Bauduin 1997
	sunlit ponds	*An. gambiae*	
	cesspits	*Cx. quinquefasciatus*	Skovmand and Sanogo 1999
	rain pubbles	*An. gambiae*	
	various polluted sites	*Cx. quinquefasciatus*	Regis et al. 2000
	various urban habitats	*Cx. quinquefasciatus*	Silva-Filha et al. 2001
Pellets/tablets	containers	*Cx. quinquefasciatus*	Lacey et al. 1984
	woodland pools	*Cx. restuans*	Lacey et al. 1988c
	sod-lined ponds	*Culex* spp.	Lord 1991
	floodwater habitat	*Ps. columbiae*	
	catchbasins	*Cx. restuans, Cx. pipiens*	Siegel and Novak 1997
Briquettes	containers (lab)	*Cx. quinquefasciatus*	Kuppusamy et al. 1987
	sod-lined potholes	*Cx. quinquefasciatus*	Lacey et al. 1988c
	ponds	*Mansonia* spp.	Pradeepkumar et al. 1988
Alginate	containers (lab)	*Cx. quinquefasciatus*	Kuppusamy et al. 1987
Microgel droplet	rice fields	*An. culicifacies*	Sundaraj and Reuben 1991
	casuarinas pits	*An. culicifacies, An. subpictus*	Sundaraj and Raghunatha-Rao 1993
Technical powder	dairy wastewater	*Cx. stigmatosoma*	Matanmi et al. 1990
	irrigated fields	*Ps. columbiae, Oc. nigromaculis*	Mulla et al. 1988b

Oliveira et al. 2003, 2004), China (Yuan et al. 2000, Oliveira et al. 2004), Thailand (Mulla et al. 2003, Su and Mulla 2004), Tunisia (Nielsen-LeRoux et al. 2002) and France (Chevillon et al. 2001, Nielsen-LeRoux et al. 2002). Resistance to *B. sphaericus* binary toxin in colonies of *Cx. quinquefasciatus* has been generated in the United States by Rodcharoen and Mulla (1994, 1996) and Wirth et al. (2000b). Using laboratory and field-collected colonies of *Cx. quinquefasciatus*, Rodcharoen and Mulla (1994) selected for resistance by challenging larvae with an LC_{80} of *B. sphaericus* strain 2362. The laboratory colony began developing resistance in the F_{20} generation [resistance ratio (RR) 8.1] increasing to 37-fold resistance by F_{80}. The field-collected colony developed an RR of 4.4 as early as F_5 increasing to 27.4 by F_{80}. Selection of a previously untreated field population of *Cx. quinquefasciatus* using an LC_{95} of *B. sphaericus* (2362), Wirth et al. (2000b) observed very rapid development of resistance. Levels of resistance increased such that F_{12} 4th instars survived toxin concentrations that were 7000 times higher than the LC_{50} of the susceptible reference colony. Rodcharoen and Mulla (1996), Wirth et al. (2000b) and Yuan et al. (2003) demonstrated cross-resistance between strains of *B. sphaericus* in colonies of *Cx. quinquefasciatus*, but not to *Bti*.

Bacillus sphaericus is at high risk for selecting resistance in mosquito populations because its binary toxin apparently binds to a single receptor type on the larval midgut microvilli. The mechanisms of resistance to the binary toxin have been elucidated by Nielsen-LeRoux et al. (1995, 1997), Silva-Filha et al. (1999), Charles and Nielsen-LeRoux (2000), Charles et al. (2000), Darboux et al. (2002), and Oliveira et al. (2004). Nielsen-Leroux et al. (1995) demonstrated that resistance in *Cx. quinquefasciatus* to the binary toxin is due primarily to a change in the receptor on midgut brush-border membranes. Subsequently, Charles and Nielsen-LeRoux (2000) and Nielsen-LeRoux et al. (2002) stated that several mechanisms are involved in resistance to *B. sphaericus*, some affecting the toxin/receptor binding step, others currently unknown, and discussed alternative assumptions regarding the dynamics of elevated resistance to the binary toxin.

Genetic analysis by Wirth et al. (2000b) revealed that *B. sphaericus* resistance in *Cx. quinquefasciatus* was inherited as a recessive trait and controlled by a single major locus. Oliveira et al. (2004) confirmed the recessive inheritance of the recessive gene. Nielsen-LeRoux et al. (2002) elucidated the genetic basis of resistance to the binary toxin in *Cx. pipiens* from France and Tunisia.

Oliveira et al. (2003) compared the biological fitness of a field-collected colony of *Cx. quinquefasciatus* that was highly resistant to *B. sphaericus* isolate 2362 (RR $>$ 163,000 after 46 generations of selection) to that of a susceptible colony originating from the same parental cohort in Pernambuco, Brazil. The resistant colony had significantly lower fecundity and fertility, and slower development than the susceptible colony. The generation time increased from 21.6 to 26 days for highly resistant generations. By contrast Rodcharoen and Mulla (1997) observed that *Cx. quinquefasciatus* larvae and pupae from females of strains resistant to *B. sphaericus* developed at slightly faster rates than those of the susceptible strains. Similar to the Brazilian study they also observed lower fecundity and fertility in resistant mosquitoes.

Regis and Nielsen-LeRoux (2000) reviewed resistance management strategies for *B. sphaericus*. They recommended monitoring the susceptibility of targeted species to *B. sphaericus* before and during treatments and promoting discontinuity of selection pressure by another or several other control agents. Alternation of *B. sphaericus* with *Bti* or use of *Bti* alone or mixtures of *Bti* and *B. sphaericus* have been studied and recommended as possible strategies to manage resistance levels (Regis and Nielsen-LeRoux 2000, Zahiri et al. 2002, Zahiri and Mulla 2003). Zahiri et al. (2004) demonstrated that colony selections using suspensions containing mixtures of commercial *Bti* and *B. sphaericus* WDG formulations did not result in resistance in *Cx. quinquefasciatus*, while selections with *B. sphaericus* WDG alone generated resistance. Mulla et al. (2003) showed a delay in development of resistance, and longer control duration against *Cx. quinquefasciatus* in the field with similar mixtures. Wirth et al. (2000c) observed that the Cyt1A toxin from *Bti* restored toxicity of *B. sphaericus* against highly resistant *Cx. quinquefasciatus*. Similarly, Wirth et al. (2001) demonstrated that the Cyt1Ab1 and Cyt2Ba1 toxins from *Bt* serovar. *medellin* and *Bti* synergize *B. sphaericus* against resistant *Cx. quinquefasciatus*. Wirth et al. (2005a) showed that evolution of resistance in *Cx. quinquefasciatus* was markedly reduced when larvae were challenged by *B. sphaericus* combined with the Cyt1A toxin from *Bti* as compared to *B. sphaericus* alone. The use of transgenic bacteria expressing toxins of both *Bti* and *B. sphaericus* has also been recommended (Federici et al. 2003, Zahiri et al. 2004, Park et al. 2005, Federici et al. this volume).

Another strain of *B. sphaericus* also shows promise. Pei et al. (2002) demonstrated that an additional protein component, naturally present in *B. sphaericus* isolate IAB59, slows the evolution of resistance in *Cx. quinquefasciatus*. They observed high resistance in their selected line in the absence of that component.

Safety to Non-Target Organisms

Laboratory and field tests against several invertebrate species confirm the specificity of *B. sphaericus* for mosquitoes and safety for the vast majority of non-targets including a variety of mosquito predators, chironomids, and other species of Nematocera (Mulla et al. 1984b, Aly and Mulla 1987, Karch et al. 1990, Lacey and Mulla 1990, Rodcharoen et al. 1991, Walton and Mulla 1991, Yousten et al. 1991, 1992; Lacey and Siegel 2000; Lacey and Merritt 2003, Brown et al. 2004). An exception is that some species of predatory mosquitoes in the genus *Toxorhynchites* may be affected under certain conditions. Larvae of *Tx. rutilus* (Coquillett), which do not feed on particulates, are unaffected by extremely high concentrations of *B. sphaericus* (2013 isolate) in the absence of prey (Lacey 1983). However, *Tx. rutilus* are killed after consuming *Ae. aegypti* larvae that have ingested the bacterium (Lacey 1983, Lacey et al. 1988b). Larvae of several other *Toxorhynchites* species were not affected by the 2013 isolate in the presence of prey larvae. Not all serotypes of *B. sphaericus* are active against *Tx. rutilus*. Second instars of this species were not susceptible to a very high concentration (10 mg/liter) of the 2297 isolate (serotype 25) in the presence of prey (Lacey et al. 1988b).

The specificity of *B. sphaericus* for mosquito larvae also completely eliminates its direct risk to vertebrates including fish, birds, and mammals (Shadduck et al. 1980, Saik et al. 1990, Siegel and Shadduck 1990a, 1990c; Lacey and Siegel 2000, Lacey and Merritt 2003).

The Mtx toxins, produced during vegetative growth of *B. sphaericus*, are less stable and specific than the binary toxin. Partridge and Berry (2002) demonstrated activity of the Mtx1 toxin against *Cx. quinquefasciatus, Ae. aegypti* and the chironomid, *Chironomus riparius* (Meigen). However, the LC_{50} for *C. riparius* (4060 ng/ml) was 81 to 214 times higher than for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. No activity was observed for *Drosophila melanogaster* or *Simulium* spp. Considering the minute amounts of the Mtx1 toxin that might accompany *B. sphaericus* formulations and its lack of stability, the risk to non-target organisms from this toxin is negligible.

Few long-term effects of repeated applications of *B. sphaericus* on aquatic community structure and diversity have been reported. Mulla et al. (1984b) and Lacey and Mulla (1990) reported no noticeable adverse effects on invertebrate fauna after season-long control of *Culex* spp. with *B. sphaericus*. Merritt et al. (2005) conducted a 3-year study in southeastern Wisconsin to assess the effects of *B. sphaericus* applied for mosquito control on non-target wetland invertebrates. The authors used 5 bioassessment measures: mean taxa richness; mean diversity; Diptera richness (minus mosquitoes); Diptera abundance (minus mosquitoes); and functional group changes in the percentage of collector-gatherers, collector-filterers, scrapers, shredders, and predators. No detrimental effects to non-target organisms could be attributed to routine application of *B. sphaericus*.

THE ROLE OF BTI *AND* B. SPHAERICUS *IN INTEGRATED MOSQUITO CONTROL*

The primary goal of the integrated vector control strategy is to reduce population densities of mosquitoes to levels at which pestiferous activity is minimized or disease transmission is reduced or interrupted with minimal negative environmental effects. Ideally, integrated control programs should comprise the most effective, complementary, and environmentally compatible interventions. Due to their efficacy and relative specificity, both *Bti* and *B. sphaericus* can be ideal control agents in integrated programs. In some situations they may provide stand-alone control, but their optimal use will be in integrated control programs where other biological control agents (fish, copepods, predator insects, parasitic nematodes, and other entomopathogens), environmental management, personal protection and the judicious use of insecticides are combined (Lacey and Lacey 1990, Lacey and Orr 1994). Their compatibility with insect biological control agents could facilitate sustained control of mosquito larvae in many situations.

The combination of *Bti* with other biological control agents has resulted in excellent initial control and in some cases prolonged suppression of mosquito larvae. Mulligan et al. (1982) reported initial control of *Cx. tarsalis* larvae in a wetlands where treatment with *Bti* was followed by longer-term abatement of larvae by insect predators. Similarly, Mulla et al. (1993) observed that populations of *Cx. quinquefasciatus*, *Cx. stigmatosoma* Dyar and *Cx. tarsalis* did not recover following treatment with *Bti* due to subsequent predation of larvae by aquatic macroinvertebrates and a reduction in ovipositional attractancy of the breeding site. Neri-Barbosa et al. (1997) demonstrated compatible use of the hemipteran predator, *Notonecta irrorata* Uhler and a slow release formulation of *Bti* extended control of mosquito larvae.

The effective combined use of larvivorous fish and bacteria has been reported by several authors. Walton and Mulla (1991), for example, reported on the successful combination of *B. sphaericus* and the larvivorous fish, *Gambusia affinis* (Baird and Girard) for control of *Cx. tarsalis*. The combination was more efficacious than either agent alone. However, although *B. sphaericus* had no effect on non-target organisms, *G. affinis* reduced populations of several

important macroinvertebrate predators of mosquito larvae. Kramer et al. (1988) demonstrated greater reduction of mosquito populations in wild rice fields that were treated with *G. affinis* and *Bti* than in fields that were only treated with *Bti* or just stocked with the fish. However, populations of non-target arthropods were significantly lower in fields stocked with *G. affinis* than in fields without fish. Other examples of the simultaneous used of fish and bacteria for mosquito control are presented by Mian et al. (1986), Bolay and Trpis (1990), Yu and Kim (1993), Yu et al. (1993), Hati (1997), Kumar et al. (1998), and Wang et al. (2000).

The combined use of bacteria with other entomopathogens has received relatively little attention. As mentioned above, alternation and/or combination of *Bti* with *B. sphaericus* has been recommended as a means of management of *B. sphaericus* resistance in *Cx. quinquefasciatus*. Although *Bti* and the fungal pathogen *Lagenidium giganteum* Couch and Bland have been evaluated side by side (Kerwin and Washino 1983, Becnel et al. 1996, Hallmon et al. 2000), their long-term combined larvicidal activity has not been assessed in the field. The oospores of *L. giganteum* can persist for several years, but may require several weeks in the aquatic environment in order to germinate and produce the infective zoospore (Kerwin and Petersen 1997). *Bti*, on the other hand, is instantly available to larvae, but has short residual activity in most natural habitats. The combination of these 2 agents or *B. sphaericus* and *L. giganteum* could thus provide long-term control of mosquito larvae in certain habitats. Laboratory experiments conducted by Orduz and Axtell (1990, 1991) on the combined effect of *Bti* or *B. sphaericus* and *L. giganteum* demonstrated that *Bti* delayed but did not prevent or reduce epizootics of the fungus in *Cx. quinquefasciatus*. Overall, the effectiveness of *L. giganteum* against *Cx. quinquefasciatus* larvae was not affected by either of the bacteria. In simulated field conditions, Orduz and Axtell (1991) made single and multiple applications of *Bti* or *B. sphaericus* in the presence of *L. giganteum* followed by drying and reflooding of the simulated habitat. The fungus persisted and reinfected larvae, and *B. sphaericus* activity persisted while that of *Bti* did not.

The use of bacteria for larval control in addition to insecticides such as pyrethroids for control of adult mosquitoes is common in the USA where biting rates are high or when transmission of mosquito-borne disease is a major consideration. Another use of insecticides that meshes well with the use of bacteria for larval control and that selectively targets indoor biting anthropophilic mosquitoes is bed nets impregnated with insecticides (Curtis et al. 2003 and several references in Lacey and Lacey 1990).

USE OF BACTERIA FOR INTEGRATED CONTROL OF MOSQUITO VECTORS OF DISEASE CAUSING AGENTS

Mosquito-borne diseases such as malaria, dengue, yellow fever, Japanese encephalitis, a variety of other arboviruses, and filariasis continue to be major public health issues in many tropical countries. The value of *Bti* and *B. sphaericus* in integrated vector-borne disease control programs has been investigated by several researchers. Xu et al. (1992) reported on the control of the malaria vector *An. sinensis* Wiedemann in Hubei Province, China using *Bti* and *B. sphaericus*. The application of the 2 microbial control agents reduced the incidence of malaria in the test area from 5.6 cases/10,000 people in 1986 before routine treatments began to 0.8 cases/10,000 people in 1989. Kumar et al. (1998) reported a decline in anopheline larvae and malaria incidence in coastal villages in Goa State, India following routine application of *Bti* and introduction of larvivorous fish. Meanwhile, the incidence of malaria in nearby towns that lacked integrated mosquito control continued to rise. Barbazan et al. (1998) reported a decrease in malaria incidence following a large-scale program in which *B. sphaericus* was applied to the wide variety of breeding sites in the city of Maroua, Cameroon. Although optimistic about the use of microbial larvicides for reducing malaria transmission, Barbazan et al. (1998) concluded that good geographic reconnaissance of breeding sites and knowledge of the vector species population dynamics is essential. Other researchers that have reported on the potential of *Bti* and/or *B. sphaericus* for control of malaria vectors are Hougard et al. (1983), Yu and Lee (1989), Perich et al. (1990), Karch et al. (1991), Sundararaj and Raghunatha-Rao (1993), Lee et al. (1994), Kroeger et al. (1995b), Hati (1997), Rivera et al. (1997), Skovmand and Bauduin (1997), and Skovmand and Sanogo (1999).

A multitude of studies using *Bti* for control of *Ae. aegypti* and other vectors of dengue have been conducted world wide. Kroeger et al. (1995a) published on the potential contribution of *Bti* in a community-based dengue control program in Colombia. Wang et al. (2000) described integrated control of *Ae. aegypti* in Taiwan. Several examples of the use of *Bti* for control of peridomestic *Ae. aegypti* and *Ae. albopictus* in areas that are endemic for dengue are listed in Table 1. Examples of the use of *Bti* and *B. sphaericus* for control of encephalitis vectors are presented by Yu and Kim (1993). The use of *Bti* and *B. sphaericus* in rice fields, breeding sites for several mosquito vectors of encephalitis viruses, was reviewed by Lacey and Lacey (1990). Several additional rice

field studies published since 1990 are listed in Tables 1 and 2.

Kumar et al. (1996) reported a sharp decline in adult and larval *Cx. quinquefasciatus* in Vasco City (Goa State, India) where Bancroftian filariasis is endemic following intensive treatment of larval breeding sites with *B. sphaericus*. Other large-scale programs for control of *Cx. quinquefasciatus* using *B. sphaericus* in areas endemic for filariasis are reported by Hougard et al. (1983, 1993) Barbazan et al. (1997), Mulla et al. (1997, 1999, 2001), Regis et al. (2000), and Silva-Filha et al. (2001).

CONCLUSION

Bti and *B. sphaericus* provide effective alternatives to broad spectrum larvicides in many situations with little or no environmental impact. Their compatibility with other biological control agents will enable a more sustainable approach to mosquito control than would be possible with conventional chemical larvicides. The comparison of biopesticides with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost. However, taking into account environmental benefits including safety for humans and other nontarget organisms, reduction of pesticide residues in the aquatic environment, increased activity of most other natural enemies and increased biodiversity in aquatic ecosystems, their advantages are numerous (Lacey et al. 2001).

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EFFECTS OF VEGETATION CONTROL ON MOSQUITOES IN SEASONAL FRESHWATER WETLANDS

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ABSTRACT. Wetland restorations benefit wildlife, but wetland vegetation may promote mosquitoes. We studied the effects of removing joint grass (*Paspalum distichum* L.) on the abundance and distribution of immature mosquitoes in seasonal freshwater wetlands in California. In 4 >4-ha plots and one 29.8-ha field, joint grass was removed via herbicide and disking. Four untreated plots and one 4.1-ha field were controls. Fields were sampled every 7–14 days in early autumn by taking 75 dips along levees, plus a 25-dip circular transect 6 m from field edges. Untreated plots had 7 times more larvae and 20 times more pupae than did vegetation-removal plots. Similar densities of mosquitoes were found along edges and within fields.

KEY WORDS *Culex tarsalis*, disking, seasonal wetlands

INTRODUCTION

After decades of wetland losses to agriculture and development, public agencies and private landowners have been restoring or creating seasonal wetlands in many areas (Collins and Resh 1989). Wetlands are being created to support bird migration and overwintering, to restore native plant and animal communities, to provide natural flood control, and to process waste water. However, restored wetlands can provide habitat for mosquitoes that vector disease-causing microbes and that potentially create social problems (e.g., decreased property values, decreased tourism, problems in hiring or retaining outdoor workers, and nuisance issues).

Certain kinds of emergent vegetation are well known to favor mosquito production (Collins and Resh 1989, de Szalay and Resh 2000, review: Jiannino and Walton 2004), whereas expanses of open water are thought to discourage mosquito production (Collins and Resh 1989). Joint grass (*Paspalum distichum* L.; also called "knot grass") is an emergent grass that grows in dense mats that can provide cover for mosquitoes. In addition, some wildlife managers regard areas covered by this grass as poor habitat for waterfowl. This study was undertaken to test whether removing joint grass would reduce mosquito numbers.

The combination of disking and herbicide treatments is a favored method for joint grass control because this grass grows back quickly after mowing and grazing. Disking has long been known as a method for reducing floodwater mosquitoes that lay their eggs in soil (Cooney et al. 1981); however, its efficacy is less well established for mosquitoes that use standing water. While it is generally believed that vegetation control will diminish mosquito production, there are surprisingly few published studies demonstrating this, especially in freshwater systems. Batzer and Resh (1992) showed that mowing vegetation in 7-m strips caused local reductions in mosquitoes in a brackish marsh, but the larvae were merely concentrating in the remaining vegetation. De Szalay et al. (1995) found that disking and mowing decreased *Culex tarsalis* Coq. and *Culiseta inornata* Williston abundances in brackish marshes, at larger scales where behavioral concentration was less likely to be important (16 × 60 m). In contrast, in a study of a wastewater treatment wetland, reducing vegetation by 50% had no effect on mosquito production from the vegetated areas, whether the vegetation was left in 5-m strips or 10-m strips. (Jiannino and Walton 2004). Open marsh water management has a history of success. However, because this technique combines vegetation reduction, pond creation, and tidal flushing in salt marshes (Dale and Hulsman 1990, Wolfe 1996), it is unclear whether vegetation reduction alone is sufficient to decrease mosquitoes.

This study evaluated whether a combined herbicide and disking treatment of joint grass infestations would help reduce mosquito production in seasonal freshwater wetlands in the Yolo Bypass Wildlife Area, a wildlife refuge 5 miles west of Sacramento, CA. Such refuges are typically flooded in late summer or early autumn to provide wetlands for migrating waterfowl.

MATERIALS AND METHODS

We established a large-scale experiment in which a 46-ha field was divided into 8 plots

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>4 ha that either had joint grass removed or were left vegetated; treatments were established in summer 2005. The large scale of the study minimized the chance that mosquito movement would bias results. In addition, we included separate disked and undisked reference fields for comparison. The main experimental field had historically high populations of both larval mosquitoes and joint grass (*P. distichum*). Within this field, we established 4 herbicided and disked areas that alternated in a checkerboard pattern with 4 untreated areas (Fig. 1). Subplots ranged in size from 4.9 to 8.1 ha. The total areas disked and undisked were similar (24.1 ha disked versus 22 ha undisked). The other 2 fields served as references. One 4.1-ha field was left untreated (Field 9). Another 29.8-ha field had been recently 75% disked and graded as part of a restoration project by the California Waterfowl Association; 30% of this field was again disked and sprayed with herbicide before the study (Field 10).

Herbicide was applied July 27 and 28 to treated plots and to the treated reference field. Joint grass was identified and treated with Roundup Pro (Monsanto Company, St. Louis, MO) at 1.14 liters per hectare, by a 757-liter (200 gallon) boom sprayer pulled by a large tractor in the treated plots and by an ATV with a 190-liter boom sprayer in the treated reference field. The majority of sites 1, 3, 5, and 7, minus islands and cattail stands, were treated with Roundup. Approximately 2 weeks after herbicide applications were made and when significant browning was observed, stubble disking was initiated with a Challenger 65E Caterpillar tractor and 4.26-m stubble disk. One to 4 passes with the disk were required depending on the density of the joint grass or other vegetation. The majority of disking was focused on joint grass, while effort was made to avoid emergent rushes, swamp timothy, and water grasses. Sites 2, 4, 6, and 8 and Field 9 were untreated controls.

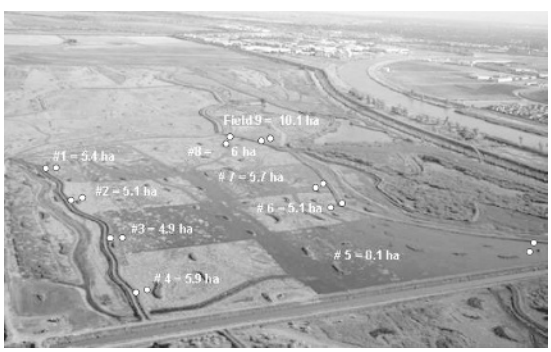

Fig. 1. Aerial photograph of wetland study plots showing sizes and locations of sites used to collect mosquito immature stages from an experiment on how vegetation removal influences mosquitoes. Field 9 was a control field; another field where vegetation was removed is not shown. Dots show approximate centers of edge transects and the beginnings of circular transects within sites.

After operations were complete, 2 researchers independently walked across each plot or field and performed visual estimates of percentage cover of joint grass and all vegetation to the nearest 10%. Estimates were always within 10% of each other.

Fields were flooded in late September and mosquito larvae were sampled from October 11 to November 17. Samples were taken along the edge and within each study site. Seventy-five dips were taken from the edge at depths of 3–6 cm, spaced ~1 m apart and we left a buffer of >20 m between plots. Twenty-five additional dips were taken within the field, by walking 6 m from the edge and taking a dip every meter in a circular pattern. Depths in these locations were 0.6–0.75 m. Each sample was taken by a standard 0.47-liter dipper and poured into a strainer for collection (mesh size 0.2 mm). A cumulative sample was taken from each edge or within-field transect. Samples were labeled and kept in alcohol until identified to taxon and growth stage. Larvae were identified to species or genus where possible. Pupae and very damaged specimens were identified to family. Edge and within-field samples were tabulated separately, but were combined for all analyses except the spatial analyses. Samples were taken every 1–2 weeks, weather permitting. We recorded pH and water temperature at each location. Temperature ranged from 25.5–10°C, decreasing as the experiment progressed, and pH was 7 in all areas.

All data were analyzed using Systat 11 (Systat Software Inc., Chicago, IL). To meet the assumptions of analysis of variance, abundance data were log transformed and percentage data were arcsin–square root transformed. Full-sized fields were treated as references rather than replicates because of differences in size.

RESULTS

The combination of herbicide application and disking was successful in eliminating joint grass in the treated experimental plots and field, and this also reduced cover of other vegetation to <10% in all sites. In contrast, the control sites had nearly 100% cover. The cover of joint grass in control plots was 20%, 40%, 40%, and 70%, and was 30% in the control field.

There were fewer immature mosquitoes (larvae and pupae of all taxa combined) in plots where joint grass was removed, and this difference increased significantly over time (Fig. 2; repeated-measures analysis of variance [ANOVA], treatment effect, df 1, 6, $F = 27.389$, $P = 0.002$; time × treatment interaction: df 4, 24, $F = 3.567$, $P = 0.021$). Averaging over the experiment, there

were 7 times more immature mosquitoes in control plots than in treated plots. Taxa collected are presented in Fig. 3 and included *Cx. tarsalis, Cs. inornata, Cx. pipiens,* and *Cs. incidens*.

Samples from the larger reference fields also showed that the vegetation-removal field had dramatically fewer mosquitoes than the control field (Fig. 4). These fields were not analyzed statistically due to lack of replication. The data from these fields are nevertheless very important because it shows that the effect of vegetation removal was consistent at the scale of whole fields.

Vegetation-removal plots produced 20 times fewer pupae than vegetated plots (Fig. 5; treatment effect df 1, 6, $F = 15.679$, $P = 0.007$). There was no detectable temporal trend. We did not perform this analysis for the reference fields because of the much smaller number of pupae found there.

Mosquito numbers were not detectably correlated with the estimated percentage of joint grass in control plots (linear regression, 2-tailed test, df 1, 2, $P = 0.48$). However, this was a very low power test since there were only 4 replicates. Vegetation-removal plots were not included in this analysis because in these plots, the absence of joint grass was conflated with the general lack of vegetation. Therefore, it would not have shown if the effect on mosquitoes was due to the exact type of vegetation.

When all taxa were pooled, there were no significant differences in numbers of immature mosquitoes sampled from the edges of plots versus those taken 6 or more meters from the edge, either across or within dates (repeated-measures ANOVA on number per dip, area effect df 1, 13, $F = 0.014$, $P = 0.907$; time by area effect df 4, 10, $F = 0.659$, $P = 0.635$). The mean number of immatures per dip averaged across dates was 0.50 ± 0.21 SE for within-field dips and 0.48 ± 0.19 SE for edge dips.

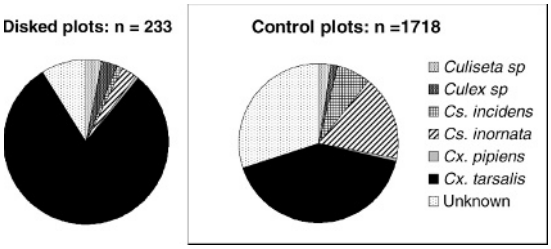

Fig. 3. Proportions of mosquito species collected in plots that either had joint grass removed (disked) or were untreated (control). The total number of immature mosquitoes collected is given as *n*. "Unknowns" were either pupae (73%) or were too small or damaged to identify.

A separate, exploratory analysis of the most abundant species, *Cx. tarsalis,* also showed no spatial effects. However, the second most abundant species, *Cs. inornata,* was approximately 4 times more abundant at the edges of control plots than within the vegetation (ANOVA on log-transformed abundances in edge versus center dips, adjusted for dip number, control plots only, df 1, 6, $F = 8.615$, $P = 0.026$). Note that vegetation-removal plots were not used in this analysis because of low numbers in both center and edge dips. Numbers of other species were too low for this analysis.

DISCUSSION

Control of vegetation through herbicide application and disking was very effective for reducing mosquito larvae in these freshwater wetlands. Vegetation-removal plots had 85% fewer immature mosquitoes and 95% fewer pupae than control plots. Batzer and Resh (1992) showed

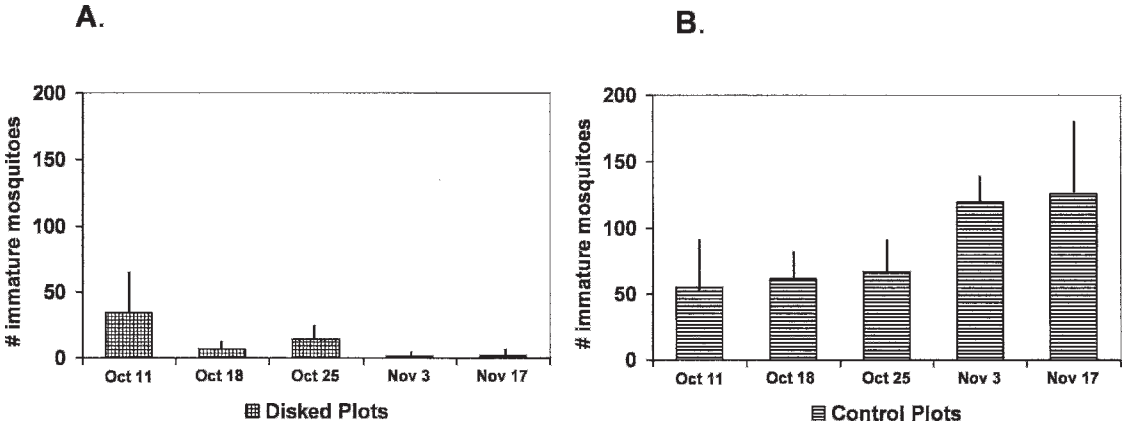

Fig. 2. Immature mosquitoes collected in plots that either had joint grass removed (disked, panel A) or were untreated (control, panel B). Bars show the means of the total numbers of mosquitoes collected in 100 dips in each of 4 plots on each date, and the standard errors of the means.

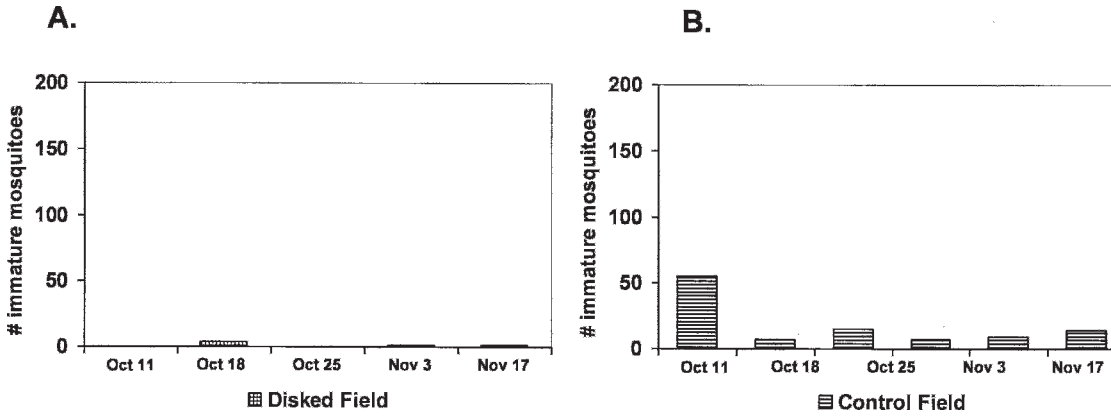

Fig. 4. Abundances of immature mosquitoes in an herbicided and disked reference field and a control field. Bars represent the total numbers of mosquitoes collected in 100 dips per field, per date.

a similar effect in a brackish marsh, where 7-m-wide mown strips of vegetation contained about 92% fewer mosquitoes than unmown strips. However, in that study mosquito numbers were elevated in the vegetated strips compared to fully vegetated plots, suggesting that the strips were concentrating the mosquitoes. The mechanism was unknown, and could have been wind, active movement by larvae, or a preference by ovipositing females for edge habitat. Walton and Mulla (1990) also found more *Cx. tarsalis* in 2-m^2 vegetated plots than in unvegetated plots. In that study plots were separated by window screening with 7 openings per centimeter, which may have restricted movement of at least larger stages. For the study presented here we cannot entirely rule out that mosquitoes were concentrated in the vegetated plots, but the multihectare scale of our study makes concentration through active movement unlikely because mosquito larvae are not strong swimmers. In addition, mosquito populations were similarly low in the reference field with vegetation removed in comparison to the untreated field, which suggests that vegetation removal reduces mosquitoes on a field-wide basis.

Comparable numbers of immature mosquitoes were collected along plot edges and within fields, therefore edge dips are sufficient to indicate overall mosquito population levels in this system. This is of great practical value for 2 reasons: it is easier to collect dips along field edges, and this method may be less disruptive to wildlife than walking out into fields. Of species that were abundant enough to be analyzed separately, *Cx. tarsalis* showed no spatial trends and can therefore be adequately sampled from field edges. However, *Cs. inornata* immatures were more abundant in edge dips in control plots, indicating that edge dips could inflate abundance estimates for this species. Other species were less abundant, and we did not have adequate power to detect their distributions. The distribution of mosquitoes in wetlands vary among species; for example Andis and Meek (1984) found that 93% of *Psorophora columbiae* Dyar and Knab immatures were found near the levees of unharvested rice

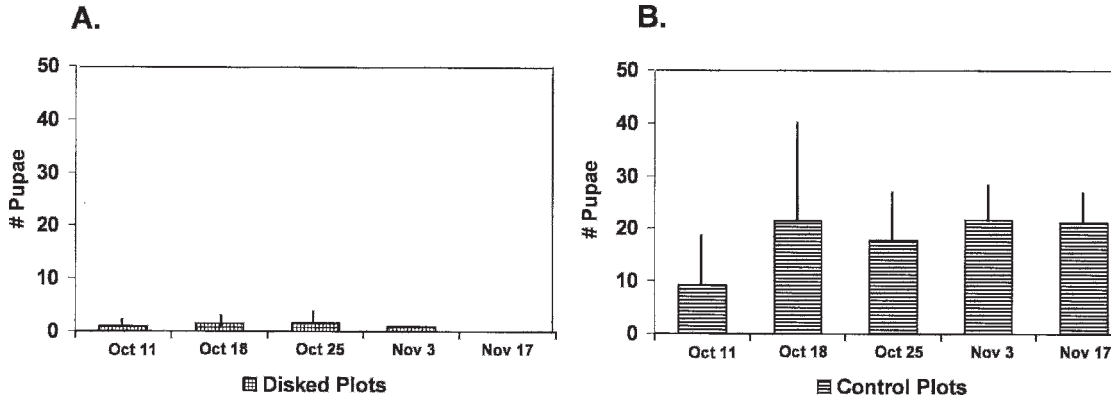

Fig. 5. Mosquito pupae collected in plots that either had joint grass removed (disked, panel A) or were untreated (control, Panel B). Bars show the means of the total numbers of mosquitoes collected in 100 dips in each of 4 plots on each date, and the standard errors of the means.

fields, whereas *Anopheles crucians* Wied. were evenly distributed across fields.

Most of the mosquitoes collected were *Cx. tarsalis,* a vector of several viruses that cause encephalitis. The second most common were *Culiseta* spp., which bite humans but are not thought to be major disease vectors. Thus, vegetation control shows good promise of reducing disease and pest problems. However, Collins and Resh (1989) caution that disking should not be performed when the ground is wet, to avoid causing depressions that might lead to poor drainage and more mosquito problems.

We were unable to establish that joint grass was correlated with mosquito production, possibly as a result of the low power of the analysis. Some studies have shown that mosquitoes may associate with particular vegetation types (Collins and Resh 1989). Pope et al. (2005) found more *Anopheles* mosquitoes in *Typha* L. (cattail) wetlands in Belize, but it was unclear whether the greater numbers were caused by the *Typha* or by agricultural enrichment of the wetlands that was associated with *Typha*. Jiannino and Walton (2004) found significantly more mosquito larvae in bulrush (*Schoenoplectus californicus* CA Meyer) than cattail (*Typha* spp.) in river water treatment wetlands. In their study, water quality was similar between vegetation types; thus, the association likely reflects effects of the vegetation itself on mosquito oviposition or survival. Further work is needed to establish the role of joint grass per se as mosquito habitat.

Although mosquito larvae and pupae were reduced in vegetation-removal areas, significant numbers of mosquitoes matured in adjacent plots. This suggests that effects of vegetation reduction were local. In some situations, local vegetation reductions can lead to greater numbers of invertebrate prey for waterfowl, while also reducing mosquitoes (review: de Szalay and Resh 2000). However, the nontarget effects of large-scale disking are poorly understood. Further research is needed to assess whether the scale of vegetation removal performed for this project affected either beneficial invertebrates, or other functions of the wetland ecosystem. Vegetation drives many ecosystem functions such as productivity, decomposition, and nutrient cycling.

ACKNOWLEDGMENTS

We thank the staffs of Sac-Yolo Mosquito and Vector Control District (MVCD) and California Department of Game and Fish (CDFG) for their hard work, and David Brown and Greg Lanzaro for facilitating the study. We thank 2 anonymous reviewers for helpful comments.

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Intensive Early Season Adulticide Applications Decrease Arbovirus Transmission Throughout the Coachella Valley, Riverside County, California

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ABSTRACT

In the Coachella Valley of California the seasonal onset of St. Louis encephalitis virus (SLEV), western equine encephalomyelitis virus (WEEV), and West Nile virus (WNV) has been detected consistently at the shoreline of the Salton Sea near the community of North Shore. The timing and intensity of initial amplification in the *Culex tarsalis* Coquillett/wild bird cycle at this focus seemed closely linked to the subsequent dispersal of virus to the rest of the Coachella Valley and perhaps southern California. In 2004, an attempt was made to interrupt the amplification and dispersal of WNV using ground ultra-low volume (ULV) applications of Pyrenone 25-5®. Although these localized treatments were started 1 month after the initial detection in April, surveillance indicated no dispersal from this focus at this time. However, these treatments appeared to have little effect, and WNV eventually was detected throughout the valley, with seven human cases reported in the urbanized upper valley near Palm Springs. In 2005, the initial detection of WNV at North Shore at the end of May was followed rapidly by dispersal throughout the valley precluding efforts at containment. Evaluation of ground and aerial applications at North Shore during May and June 2005, respectively, indicated variable kill of sentinel mosquitoes (overall mortality: ground, 43%; air, 34%) and limited control of the target *Cx. tarsalis* population. In 2006, aerial ULV applications with the same chemical were begun immediately following the first detection of virus in mid-April, resulting in an apparent reduction of *Cx. tarsalis* abundance and delay of WNV activity in the rural lower valley and a marked decline in transmission by *Culex quinquefasciatus* Say populations in the densely populated upper northwestern valley with no human cases reported. Key Words: Adulticide—Control—*Culex tarsalis*—*Culex quinquefasciatus*—West Nile virus.

INTRODUCTION

Our research addresses the hypothesis that intensive early season mosquito control directed at a critical nidus of early season virus amplification may interrupt or delay amplification, limit dispersal, and thereby preclude outbreaks of disease. Increasing early season mosquito mortality previously was shown to have a marked impact on the resulting summer population using a compartmental simulation model (Moon 1976), but the success of this approach has not been demonstrated in the field.

The on-going epidemic of West Nile virus (WNV) in Coachella Valley has provided a unique opportunity to test our hypothesis, because the seasonal onset of transmission in southern California frequently begins in late spring or early summer at a small wetland near the northeastern shore of the Salton Sea (Reisen et al. 1992). Historically, St. Louis encephalitis virus (SLEV) and western equine encephalo-

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myelitis virus (WEEV) dispersed north and westward from this focus to the remaining shoreline and then throughout the agricultural regions of the southern valley (Reisen et al. 1995a,b) as summarized conceptually in Figure 1. This dispersal appeared to be driven by the abundance gradient of *Culex tarsalis* Coquillett that declined progressing northward and upland away from the Salton Sea (Reisen and Lothrop 1999). Although *Culex quinquefasciatus* Say from this area is a potential vector of SLEV (Meyer et al. 1983), it has not been incriminated in the transmission of SLEV in the urbanized upper valley. However, with the invasion of WNV, urban *Cx. quinquefasciatus* have assumed greater importance (Reisen et al. 2004) and required intensive surveillance and control.

Since its introduction in 2003, WNV activity has been initiated at the same early season focus during 2004, 2005, and 2006 (Reisen et al. 2008). This pattern of focal early transmission and apparent dispersal provided an opportunity to disrupt focal virus amplification through intensive early season mosquito control, because the North Shore focus lies at the extreme southeast corner of the Coachella Valley, with only a narrow corridor of wetlands and agriculture connecting this area to the rest of the valley (Fig. 1). The current study documents the impact of reactive ground, and proactive ground and aerial control by the Coachella Valley Mosquito and Vector Control District (CVMVCD) on the amplification of WNV at North Shore and dispersal to the rest of the Coachella Valley.

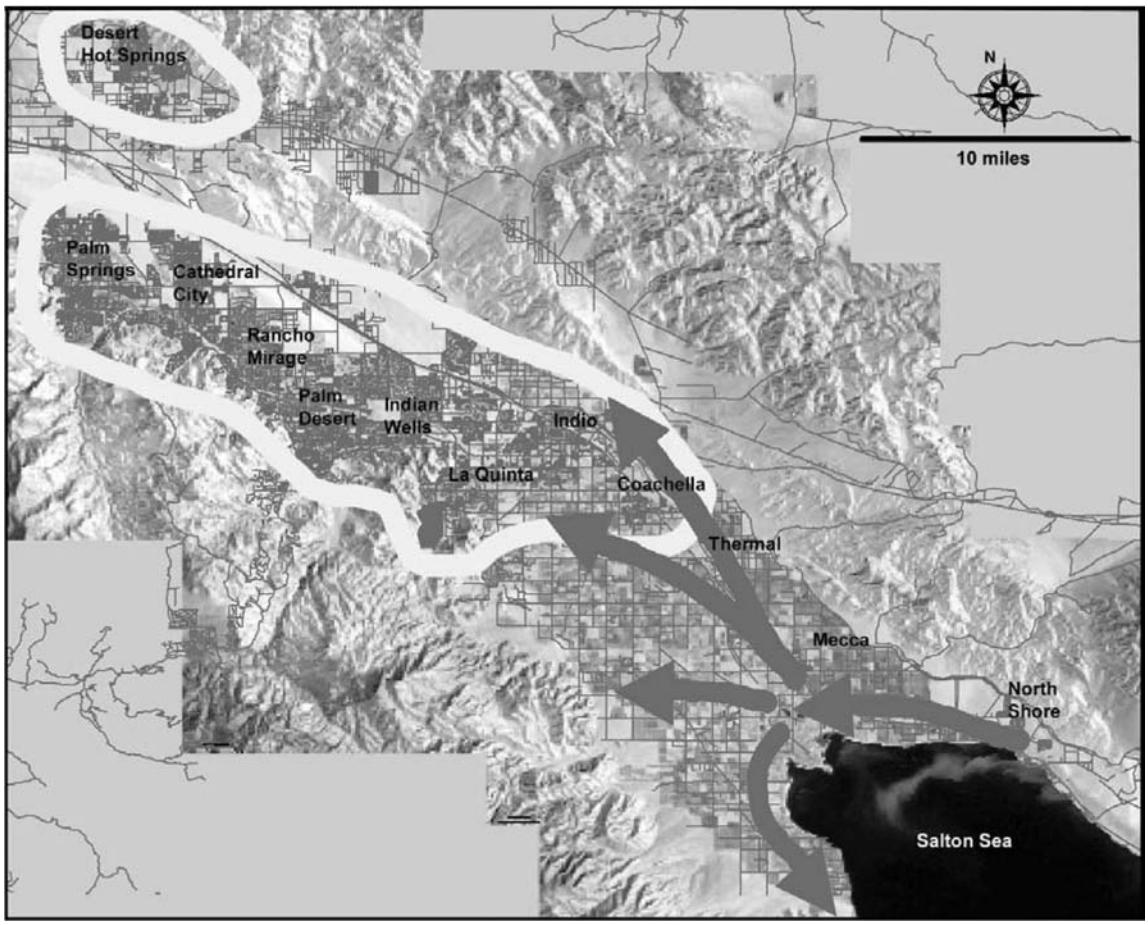

FIG. 1. Pattern of West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) dispersal from North Shore in *Culex tarsalis* (depicted by gray arrows). Area of WNV infection in *Cx. quinquefasciatus* in urban areas depicted by white perimeter.

METHODS

Surveillance

Mosquito abundance and infection was monitored in Coachella Valley by 58 dry ice–baited CDC-style traps (CO2T) (Newhouse et al. 1966) run biweekly, nine sentinel chicken flocks each comprised of 10 sentinel hens (Lothrop et al. 1992) sampled biweekly, and 17 gravid traps (Cummings 1992) run weekly in the upper valley during 2005 and 2006 (Fig. 2). Six additional CO2Ts and three sentinel flocks of 10 hens each were maintained in Imperial County along the southern shore of the Salton Sea to monitor virus at wetland habitats at or near wildlife refuges. These refuges are relatively similar to the northern shore of the Salton Sea in Coachella Valley, rarely were treated for larval or adult mosquito control during the 2004–2006 period, and therefore provided a useful negative comparison area to intensively treated sites in Coachella Valley. CO2Ts primarily monitored *Cx. tarsalis,* which was the predominant vector species in rural areas, whereas gravid traps targeted *Cx. quinquefasciatus* in the urban upper valley. Sentinel chicken flocks upland from the margin of the Salton Sea were accompanied by two CO2Ts each that have been relatively successful in collecting *Cx. quinquefasciatus* compared to gravid traps in the same areas (Reisen et al 1999). Additional CO2Ts were run intermittently during various research projects, locally enhancing arbovirus surveillance sensitivity. Although the start dates for surveillance varied slightly among years, multiple negative samples were collected each year prior to the detection of WNV activity.

Mosquitoes were returned to the CVMVCD, where they were anesthetized with triethylamine, enumerated by species, most pooled into lots of 50 females each, and frozen at −80°C. Pools were shipped on dry ice to the Arbovirus Unit at the Center for Vectorborne

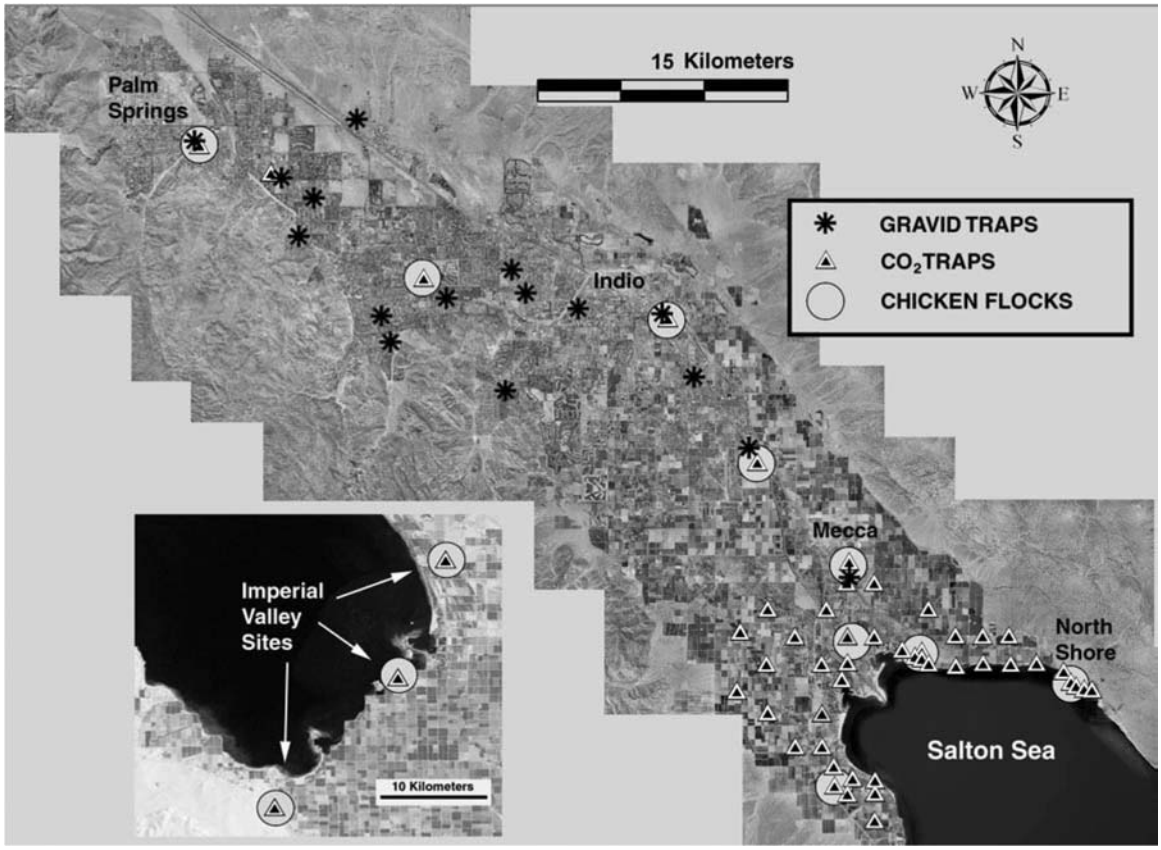

FIG. 2. Surveillance sites in the Coachella and Imperial Valleys, 2003–2006. Gravid traps were only deployed during 2005–2006.

Diseases (CVEC), where they were tested concurrently for WEEV, SLEV, and WNV using a multiplex real-time reverse transcription—polymerase chain reaction (RT-PCR) assay (Brault et al., unpublished data). Trap counts were transformed by ln(y + 1) for analyses, and abundance was expressed as backtransformed or geometric mean number of females per trap-night (Reisen and Lothrop 1999). Mosquito infection rates were estimated by a maximum likelihood method to account for variation in pool size (Biggerstaff 2003). Sentinel chickens were bled by lancet stick of the comb (Reisen et al. 1993), with blood specimens screened for antibody against WEEV or SLEV/WNV by enzyme immunoassay (EIA) and confirmed; the infecting virus was identified by plaque reduction neutralization assay (PRNT) by the Viral and Rickettsial Diseases Laboratory of the California Department of Health Services (Hom et al. 2004). Positive sentinel chickens were replaced to maintain sensitivity. Infections were expressed as the proportion of negative chickens seroconverting per 2-week interval. Protocols for the care and bleeding of sentinel chickens were approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

Dead birds reported by the public to the Dead Bird Hotline (McCaughey et al. 2003) were shipped to CVEC where oral swabs and/or kidney tissues were tested for WNV RNA using standard RT-PCR methods (Kauffman et al. 2003). Because of the low numbers of corvids present in Southeastern California (Reisen et al. 2006), relatively few birds were tested annually.

Human cases were detected passively by medical providers in Coachella Valley, and were reported to Riverside County Health Department and to the Viral and Rickettsial Diseases Laboratory of the California Department of Public Health.

Control

Although our report focuses on adulticide applications, the CVMVCD for the past decade also has attempted to suppress mosquito populations in the North Shore area with a larviciding program using VectoBac® (Valent BioSciences, 870 Technology Way, Libertyville, IL) and the growth regulator Altocid® (Wellmark International, Schaumburg, IL). The arrival of WNV necessitated enhanced suppression leading to the adulticide applications reported in the current paper. Ground applications were done by a truck-mounted Pro-Mist® ultra-low volume (ULV) fogger (Clarke Mosquito Control Products, Inc., Roselle, IL) using undiluted

TABLE 1. *CX. TARSALIS* INFECTION RATE AT RURAL NORTH SHORE, *CX. QUINQUEFASCIATUS* INFECTION RATE IN THE URBANIZED UPPER VALLEY, AND OVERALL INFECTION RATES FOR COACHELLA VALLEY, ESTIMATED BY THE MAXIMUM LIKELIHOOD METHOD (BIGGERSTAFF, 2003)

		Cx. tarsalis		*Cx. quinquefasciatus*	
		North Shore	*Total*	*Upper Valley*	*Total*
2004	Pools Tested	244	1153	147	230
	WNV positive	37	92	2	4
	IR	5.35	2.01	0.59	0.76
	LL	3.83	1.62	0.11	0.25
	UL	7.70	2.45	1.92	1.81
2005	Pools Tested	499	2198	475	662
	WNV positive	11	39	53	54
	IR	0.50	0.42	4.19	2.92
	LL	0.27	0.31	3.18	2.22
	UL	0.88	0.57	5.44	3.78
2006	Pools Tested	293	1823	689	930
	WNV positive	4	36	2	2
	IR	0.37	0.47	0.31	0.07
	LL	0.12	0.34	0.13	0.01
	UL	0.88	0.64	0.64	0.24

IR, MLE infection rate per 1,000; UL and LL, upper and lower 95% confidence limits of the IR estimate.

TABLE 2. MEAN SENTINEL MORTALITY DURING GROUND APPLICATIONS OF PYRENONE 25-5 DURING MAY 2005 AT THE COMMUNITY OF NORTH SHORE, COACHELLA VALLEY

Cluster	*n*	*24-May*	*26-May*	*28-May*	*Mean*
NW	9	65	55	18	46a
Mid	6	26	47	0	24b
SE	12	59	41	34	49a
Mean	27	59a	47a	21b	43

Means followed by the same letter were not significantly different ($p > 0.05$) using a Fisher's LSD test, n = number of cages in each cluster.

Pyrenone 25-5® (Bayer Environmental Science, Montvale, NJ) at the rate of 0.0025 lb/acre of active ingredient. Routes were designed to follow all available roads to give maximum coverage. Aerial applications were done by a single-engine, fixed wing aircraft (Air Tractor 301, Air Tractor Inc., Olney, TX) equipped with two Micronair® AU5000 atomizers (Micron Sprayers Ltd., Bromyard Industrial Estate, Bromyard, Herefordshire, UK). Pyrenone 25-5 was diluted at a ratio of 1:2 with BVA Spray 13® oil (BVA Inc., Wixom, MI) to minimize evaporation and aid in droplet descent (Lothrop et al. 2007b).

Ability of the CVMVCD to effectively disperse spray droplets into the target treatment area by ground and aerial ULV applications was assessed by bioassay during 2005 using replicated sentinel cages (Townzen and Natvig 1973) containing 15–25 mosquitoes positioned

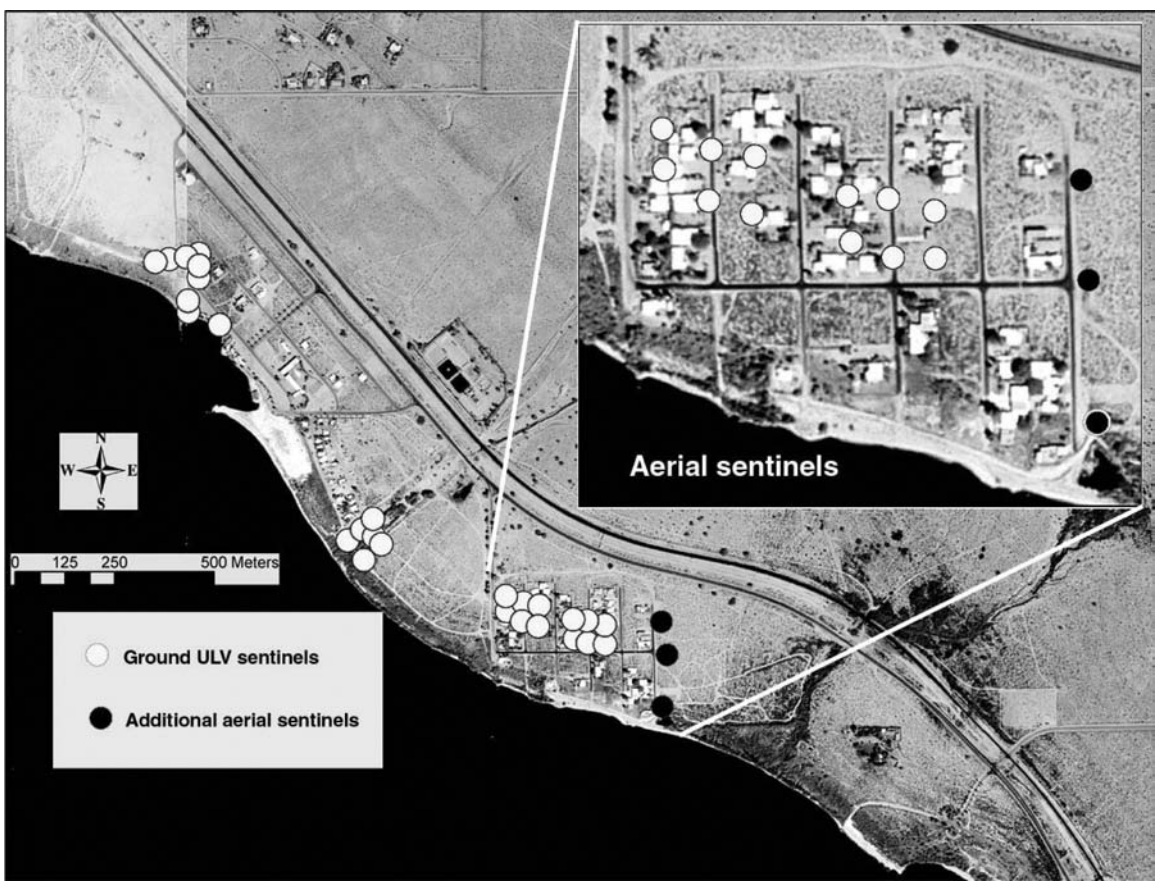

FIG. 3. Sentinel mosquito cage distribution for the evaluation of ground and aerial ultra-low volume (ULV) applications in 2005.

at 1–3 m in height (Lothrop et al. 2007a) and by impingement of droplets on 25 mm × 75 mm Teflon®-coated glass slides rotated by a Bio-Quip® (Rancho Dominguez, CA) Aerosol Droplet Sampler ("slide spinner"). Droplets were counted in size ranges of ≤40 μ, 41–70 μ, 71–100 μ, and >100 μ. For the ground ULV assessment in May 2005, sentinels and slide spinners were clustered at three sites within the treated area (Fig. 3) to sample droplet penetration through representative landscapes. The NW cluster was set in low desert scrub, the middle cluster was set along a *Tamarix* stand within a dry wash, and the SE cluster was deployed along four transects through a small residential community at Mecca Avenue. Slide spinners were distributed among the sentinel clusters. The aerial trial was done in the same region in June 2005, but the sentinel transects and two slide spinners were deployed only within the small community, and downwind and toward the shoreline vegetation.

RESULTS

Culex tarsalis abundance at 14 CO2Ts operated biweekly at North Shore varied markedly among the 4 years of monitoring (Fig. 4A). Overall vernal geometric means during 2003, 2004, and 2005 (38.8, 34.7, and 41.1 females per trap night, respectively) without intensive adult control were significantly greater than during 2006 (27.8 females per trap night, respectively) when intensive adulticide operations were conducted (F = 2.92, df = 3, 204, p =

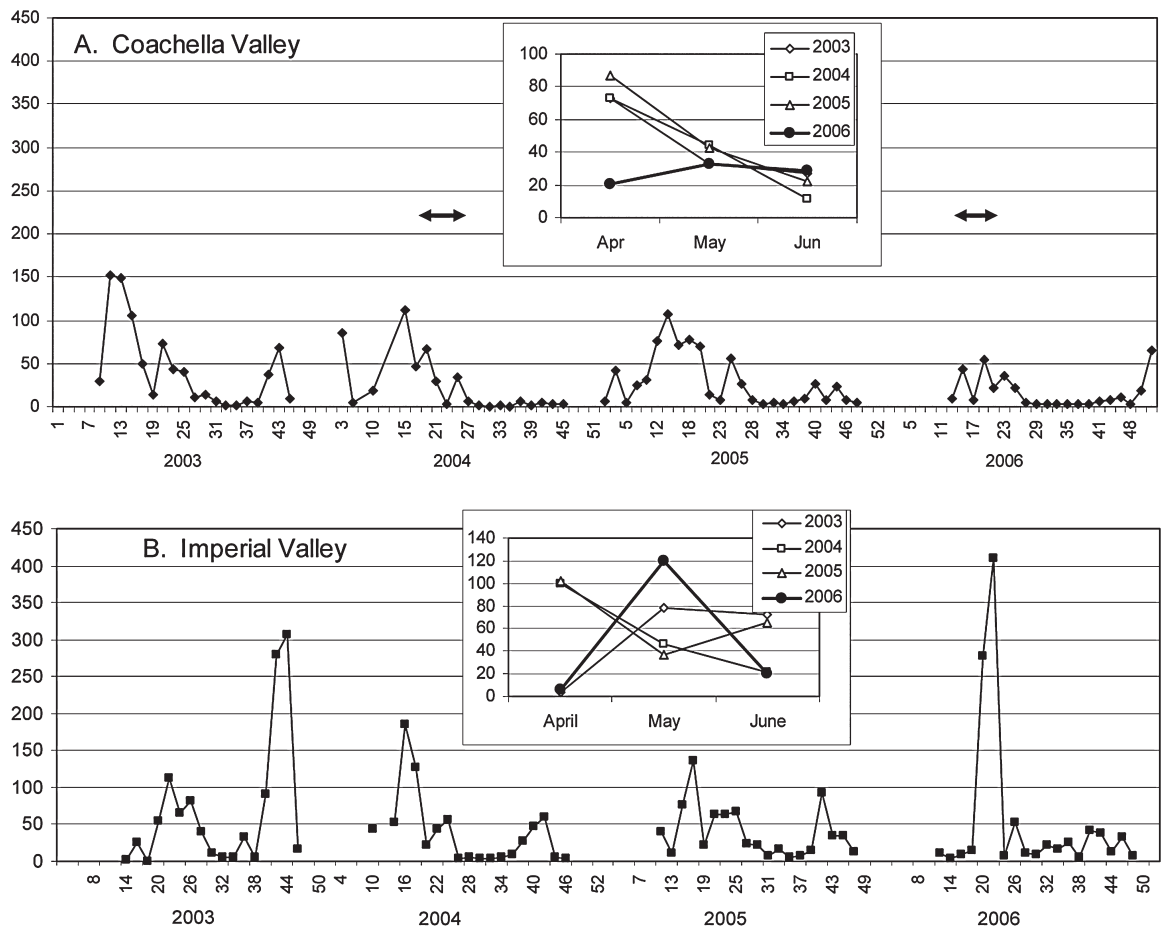

FIG. 4. Geometric mean number of *Cx. tarsalis* collected per CO2T night at North Shore area, Coachella Valley **(A)** and marshes at the southern shore of the Salton Sea, Imperial County **(B)**. Arrows show the periods of intensive ground (2004) and aerial plus ground (2006) adulticide applications. Geometric monthly means during April–June are shown in the insets.

0.04). These means were markedly lower than observed historically at North Shore (Reisen et al. 2002), when the Salton Sea peaked at −266.75 feet below sea level, peripheral marshes were more extensive, and before intensive springtime larval control using Vecto-Bac and Altocid pellets was initiated. Differences among years were attributed to the impact of adulticiding during April (Fig. 4), because the month by year interaction effect was significant ($F = 4.48$, df = 6, 204, $p < 0.001$) when tested by a three-way analysis of variance (ANOVA) with year, month and sites as main effects. In addition, *Cx. tarsalis* abundance at six CO2Ts in Imperial County did not show a significant reduction in abundance during 2006 ($F = 1.97$, df = 3, 73, $p > 0.05$) and even though there was a significant month by year interaction ($F = 5.52$, df = 6, 73, $p < 0.001$), May 2006 had the highest vernal mean (Fig. 4B).

2003

WNV invaded Imperial County in July and then Coachella Valley in August (Table 1). No human cases were reported, and low-level enzootic transmission seemed confined to rural areas in the lower valley at the Salton Sea. WNV was not detected in 275 pools of *Cx. quinquefasciatus* or sentinel chickens at upper valley sites.

2004

Despite extensive efforts to contain WNV following the early spring detection on 14 April, virus dispersal during 2004 followed the historical pattern shown in Fig. 1. Although mostly confined to the lower valley in *Cx. tarsalis* (Table 1), there was low level infection detected in *Cx. quinquefasciatus*, seroconversions at sentinel chicken flocks, and seven hu-

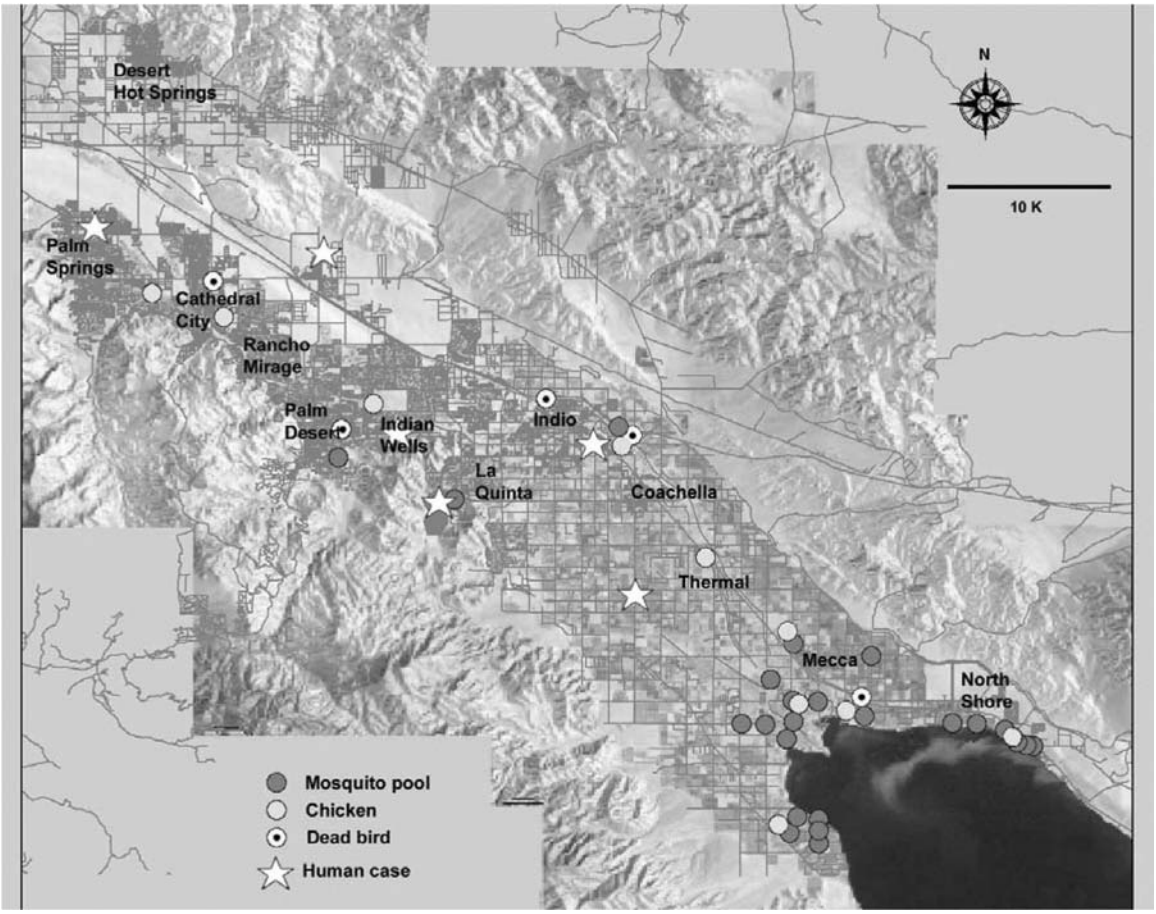

FIG. 5. Distribution of positive mosquito pools, sentinel chicken flocks, dead birds, and human cases, Coachella Valley, 2004.

man cases in the upper valley by the end of September (Fig. 5). Ground ULV applications at North Shore began on 17 May (Fig. 6), one month after initial detection of WNV on 14 April. Although all WNV activity appeared to remain contained near the North Shore focus during the month between detection and intervention, ground applications over the next 56 nights failed to interrupt WNV transmission at this site or elsewhere throughout most of the Coachella Valley. Gravid traps were not run routinely in the upper valley at this time; however, positive pools of *Cx. quinquefasciatus* collected at CO2Ts were detected in the upper valley in association with the seven human cases (incidence of approximately 2.18 cases per 100,000).

2005

WNV was first detected at North Shore on 29 May, 1 month later than in 2004, and then rapidly appeared in the *Cx. quinquefasciatus* population in the urbanized upper valley (Fig. 7), preventing any attempt at containment. Extensive gravid trapping was initiated in the upper NW portion of the valley (Fig. 2), increasing the sensitivity of *Cx. quinquefasciatus* sampling, the number of pools tested to 662, and the infection rate to 4.19 per 1,000 (Table 1). Midseason enzootic activity, July–September, in the upper valley was widespread, with *Cx. quinquefasciatus* infection rates as high as 34 per 1,000 in one residential neighborhood. Reactive ground adulticide treatments were made at sites throughout the valley with elevated mosquito infection rates. Widespread distribution and high mosquito infection rates in the upper valley were associated with five reported human cases (incidence, 1.56 per 100,000), comparable to 2004.

Reactive adulticide applications at North Shore provided the opportunity to asses the operational efficacy of ground and aerial ULV

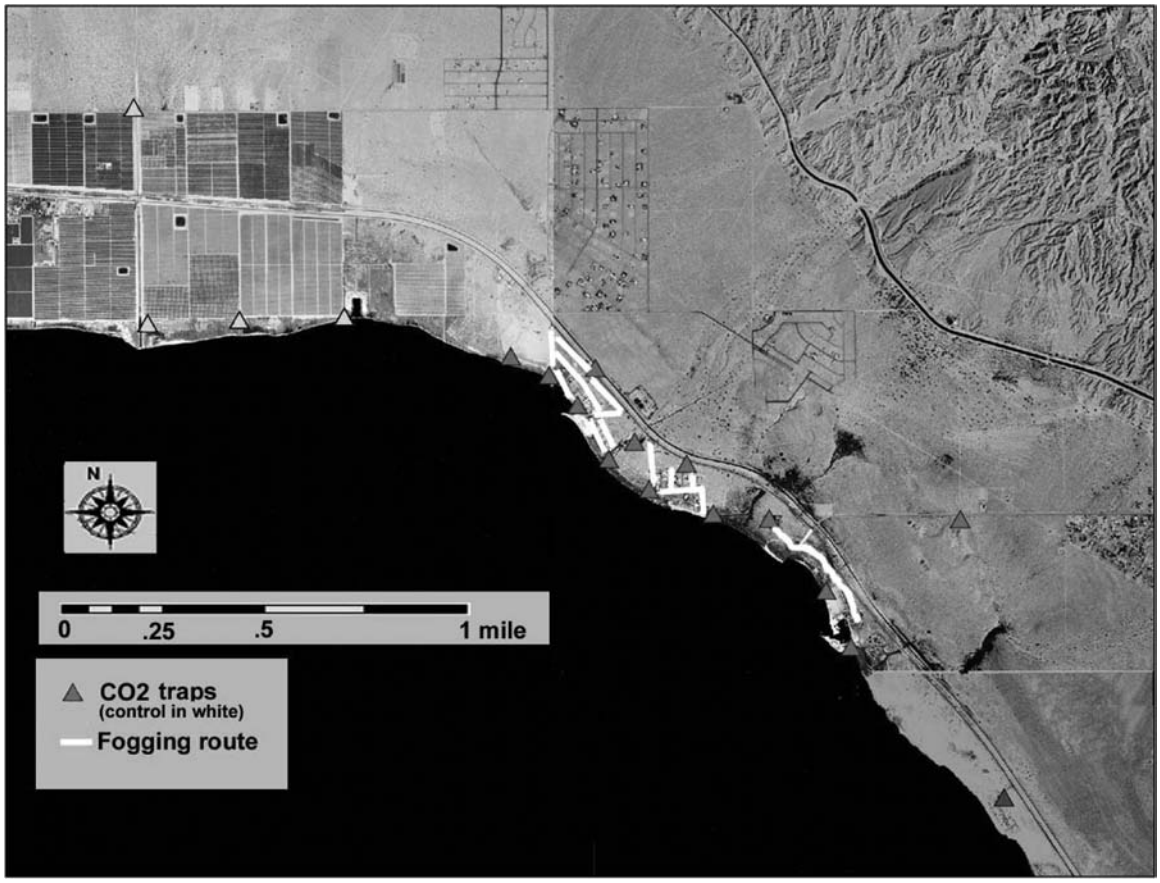

FIG. 6. Route for ground ultra-low volume (ULV) treatments at North Shore, 2004.

applications. Three alternate nights of ground ULV applications of Pyrenone 25-5 in May 2005 at North Shore provided variable sentinel mortality at three sites along the Salton Sea shoreline (Table 2). Overall, 43% of 2,786 *Cx. tarsalis* sentinels in 27 cages were dead at approximately 12 h after application. Mortality following treatments 1 and 2, when NW winds were 1 and 3 mph, respectively, was significantly greater ($F = 11.2$, $df = 2, 72$, $p < 0.001$) than during treatment 3, when the winds were 8–10 mph from the SE. During treatments 1 and 3, winds carried the fog to sentinels along the shore to the SW and into the small community at Mecca Avenue, but not into the *Tamarix* along the wash, at the central cluster of cages (Fig. 3), where significantly lower sentinel mortality was recorded ($F = 5.4$, $df = 2, 72$, $p = 0.007$), and not to the majority of shoreline vegetation, where the natural population tends to be concentrated (Lothrop et al. 2002). The Mecca Avenue community consisted of small houses with low and relatively open desert landscaping, allowing effective particle dispersal for at least 1.5 blocks (130 m) from the truck route, although efficacy varied over time among replicate applications. There were significant differences ($F = 11.2$, $df = 4, 54$, $p < 0.001$) among abundance of *Cx. tarsalis* at traps within the different treatment zones (Fig. 9), but these differences in abundance remained consistent over time, because both the time and interaction effects were not significant ($p > 0.05$), indicating that the spray did not alter abundance. In agreement, percentage control calculated using the formula of Mulla et al. (1971) was negative, indicating no control. Failure to detect a significant suppression of abundance may have been associated with

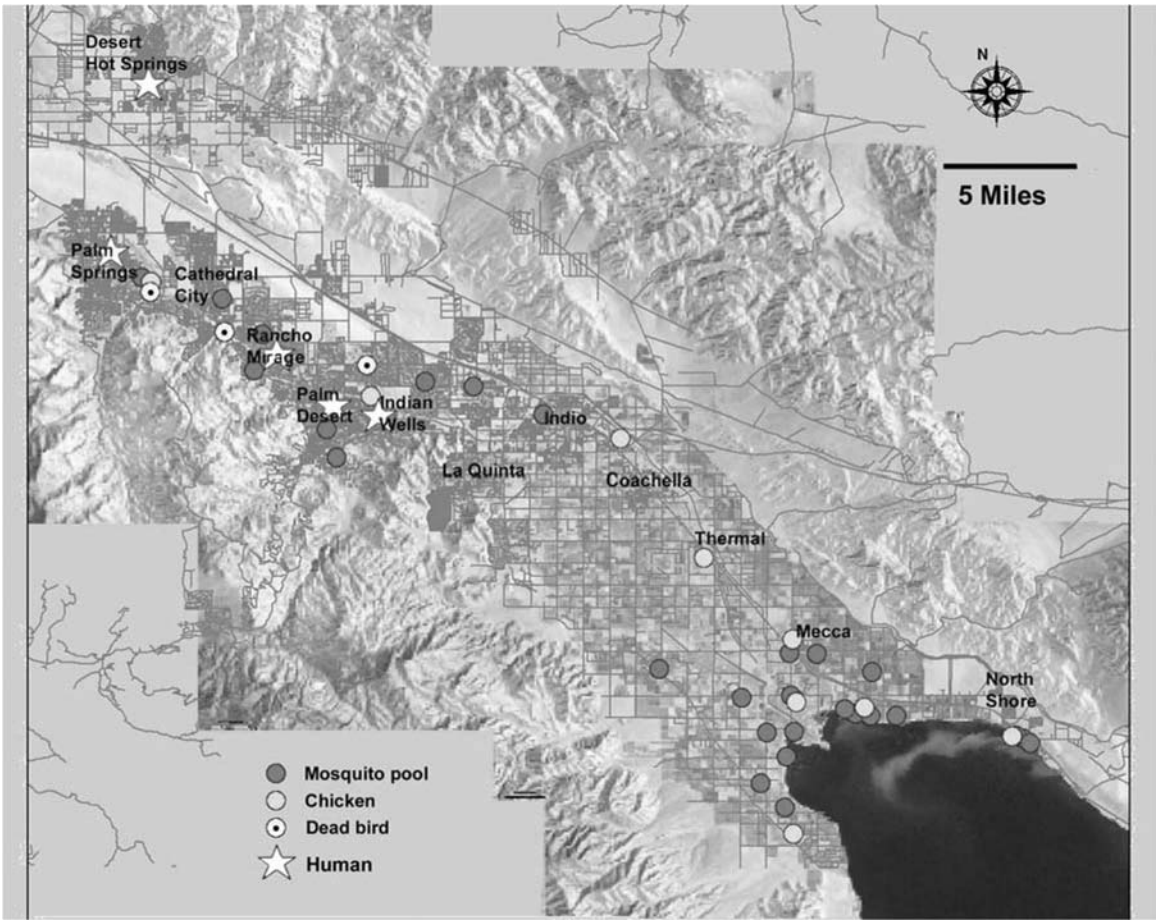

FIG. 7. Distribution of positive mosquito pools, sentinel chicken flocks, dead birds, and human cases, Coachella Valley, 2005.

rapid replacement of adults emerging from nearby wetland habitats.

Three consecutive nights of aerial treatment at North Shore in June 2005 were assessed by five CO2Ts distributed along the shoreline and 16 cages of mosquito sentinels positioned within the Mecca Avenue community. Mortality among these sentinel cages was more variable than observed at the same sites during the May ground treatment. Overall, 34% of 1,132 mosquitoes deployed in 16 cages on 3 nights were dead 12 h after spray, with means of 12–49% over the 3 nights of treatment and 0–97% among cages on the same night. There were distinct gaps in coverage, where the particle cloud apparently missed the target area during spray 1 and produced minimal mortality during sprays 2 and 3. There was no significant ($p > 0.05$) reduction in abundance comparing *Cx. tarsalis* catch 2 nights before to 2 nights after treatment: mean abundance of *Cx. tarsalis* per CO2T night in the core area decreased from 20 to 14 females, while the controls decreased from 163 to 99 females per CO2T night. Percent control using Mulla's formula was estimated to be 8%.

2006

The failure of reactive ground applications in 2004 and the surprisingly rapid spread of WNV in 2005 prompted the CVMVCD to implement proactive aerial and ground ULV treatments at North Shore at the end of April 2006 after WNV was detected (Fig. 10). Although the aerial trials at North Shore in 2005 had disappointing results, the dependence of ground applications on access roads and prevailing winds and the ability to cover large areas by air, during the period of maximum host seeking just after sunset (Reisen et al. 1997), supported the decision to use aerial applications. The initiation of treatment was based upon an average abundance of >70 *Cx. tarsalis* in CO2Ts in and around North Shore, which was considered a risk for rapid WNV amplification. From 24 April through 2 June, ULV adulticides were applied by ground and air. Ground applications covered 26 hectares inside the Salton Sea Stare Park on each of 31 treatments during this period. Aerial applications covered 150 hectares for the first seven treatments, were extended westward to cover 180 hectares for the next seven treatments, and then continued on the western 78 hectares of the overall treated area for the last 12 treatments. Adjustments to the course and height of the flight path used in 2005 were made to better target the upland edge of shore-

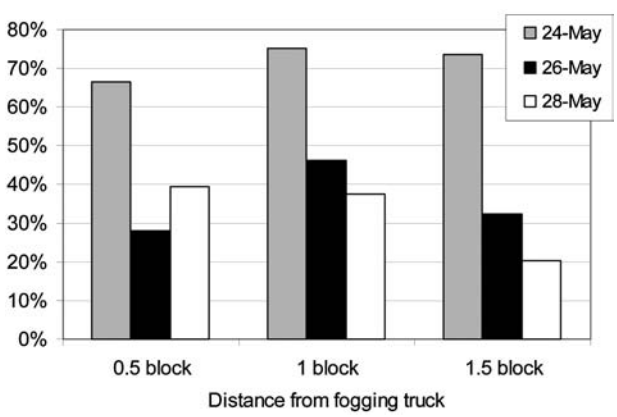

FIG. 8. Mean mortality of sentinel mosquitoes along four transects within the Mecca Avenue community as a function of sentinel cage distance from the ultra-low volume (ULV) ground application truck route.

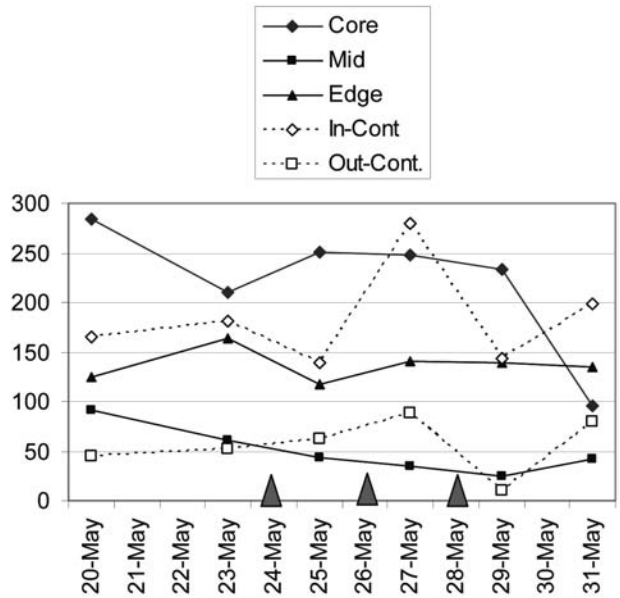

FIG. 9. Geometric mean abundance of *Cx. tarsalis* females trapped at replicate CO2Ts located in the core or center, middle, and edge of the area treated by ground equipment with ultra-low volume (ULV) adulticide and at CO2Ts placed 1 (in) and 2 miles outside (out) the treated area, May 2005. Triangles show nights of treatment.

line vegetation. Slide spinners were placed along this line of vegetation to document droplet dispersal at an area where prevailing winds tended to carry the droplets away from the target area. This was considered to be the most difficult area to reach and was used as the worst case scenario. As with all our aerial trials, due to landscape and atmospheric influences, there were gaps in coverage as measured by droplet impingement and/or sentinel mortality. The average densities of droplets of <40 μ in diameter on slides at five spinners on 4 nights sampled during April ranged from 0 to 275 droplets per cm^2, documenting variability among sites and applications. Mortality for sentinel mosquitoes deployed in 10 cages on 26–27 April averaged 24%, ranging from 0% to 86%. Percent control for *Cx. tarsalis* abundance based on CO2Ts operated pretreatment and on 26 April, 11 May, and 25 May post-treatment was estimated to be an encouraging 70%, 45%, and 69%, respectively.

Results from mosquito pools collected in April just prior to treatment showed that applications had begun just after the onset of WNV activity and that dispersal out of this focus may have been in progress. The CVMVCD responded by extending the treatment area further west to stay ahead of the presumed path of dispersal. Primary emphasis was placed on aerial ULV treatments along the shoreline with ground ULV filling in areas such as the Salton Sea State Park to the southeast of North Shore (Fig. 10). These treatments were concluded when results from surveillance indicated the continued absence of virus activity. Although WNV appeared at the North Shore focus and began to spread westward, it did not follow the expected seasonal pattern (Fig. 1) and was detected in only one sentinel chicken and two *Cx. quinquefasciatus* pools in the upper valley (Fig. 11 and Table 1). Late season activity was limited to the lower valley and primarily associated

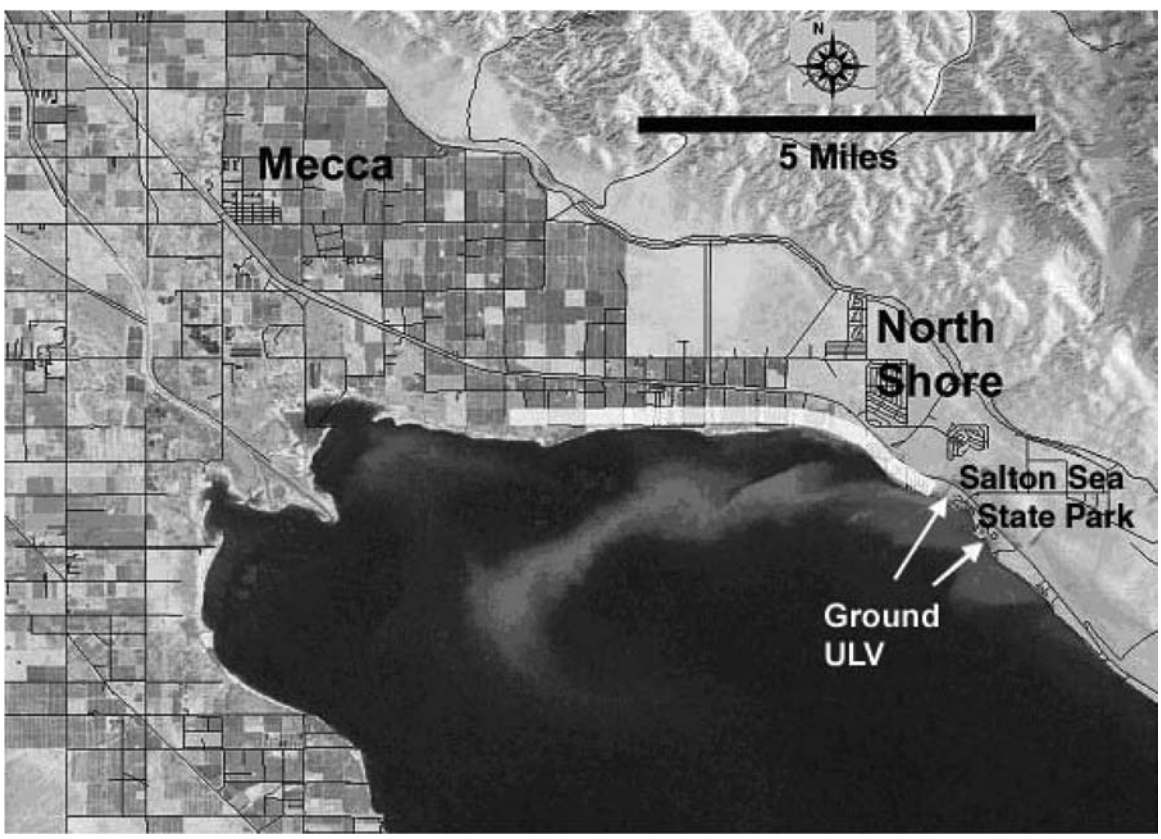

FIG. 10. Area treated by ground and aerial ultra-low volume (ULV) along the northern shore of the Salton Sea during 2006. Aerial spray swath is shown in white.

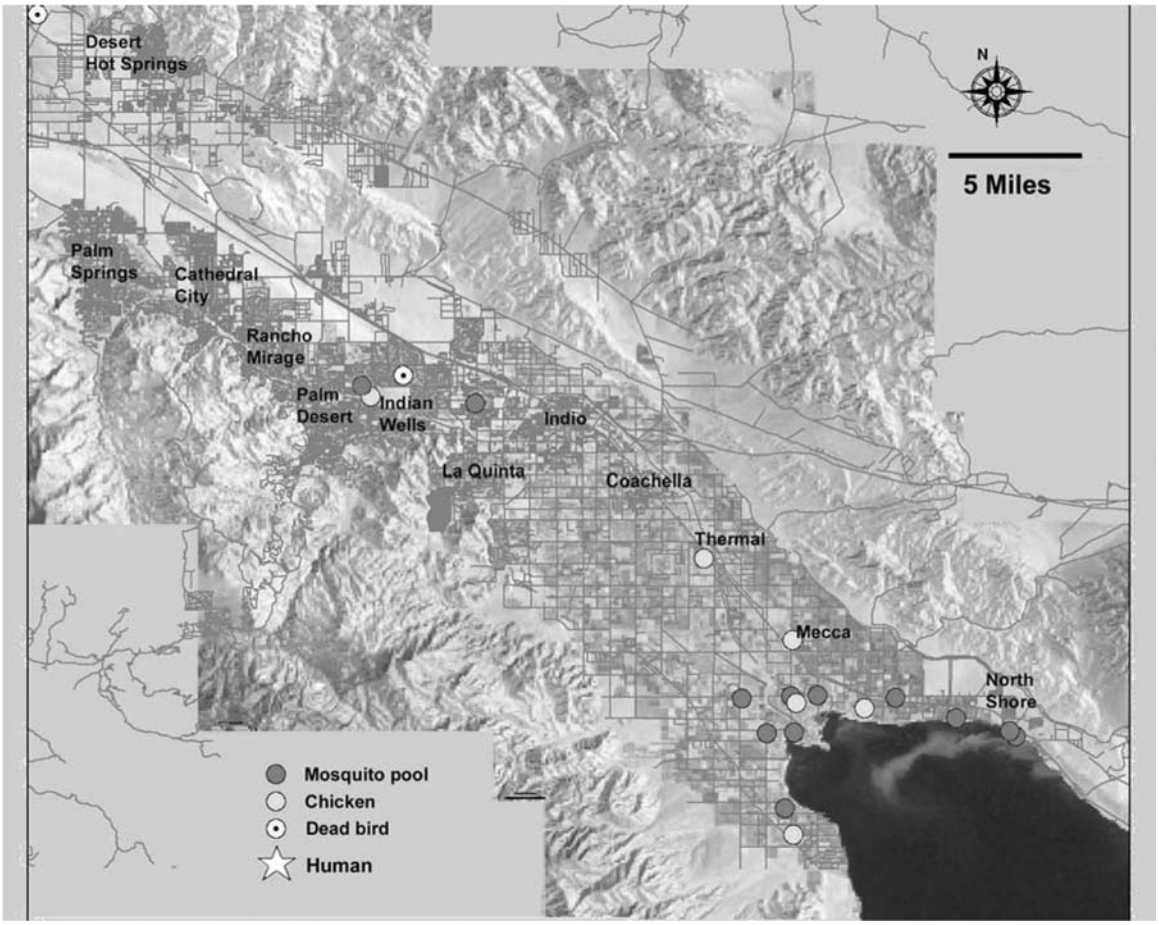

FIG. 11. Distribution of positive mosquito pools, sentinel chicken flocks, dead birds, and human cases, Coachella Valley, 2006.

with duck club flooding and local increases in *Cx. tarsalis* abundance (Fig. 11).

DISCUSSION

Effective vernal suppression of *Cx. tarsalis* population abundance at North Shore appeared to effectively delay WNV amplification and subsequent dispersal to the rest of Coachella Valley. Parameters used to assess the interruption of virus transmission included seroconversion rates among sentinel chickens and infection rates in *Cx. tarsalis* and *Cx. quinquefasciatus* mosquitoes (Figs. 12 and 13). Too few dead birds or human cases were detected for meaningful statistical analyses. Figures 12 and 13 summarize all surveillance sites in the Coachella Valley and three sites in the Imperial Valley. WNV initially entered the Coachella Valley in August 2003, but remained limited to the lower valley (Reisen et al. 2004). The first full season of WNV activity was during 2004, and although WNV enzootic activity was most intense within the *Cx. tarsalis* populations in the rural lower valley, the seven human cases in the upper valley point to *Cx. quinquefasciatus* involvement, because *Cx. tarsalis* is largely absent from this area. These results indicated a failure to contain WNV at its North Shore focus using reactive ground ULV adulticiding. Although evaluations of ground trials in 2005 (Lothrop et al. 2007a) indicated that this method can effectively reduce abundance, the dependence on wind to disperse the fog and the unfavorable direction of the prevailing winds may have prevented the droplets from reaching large portions of *Cx. tarsalis* popula-

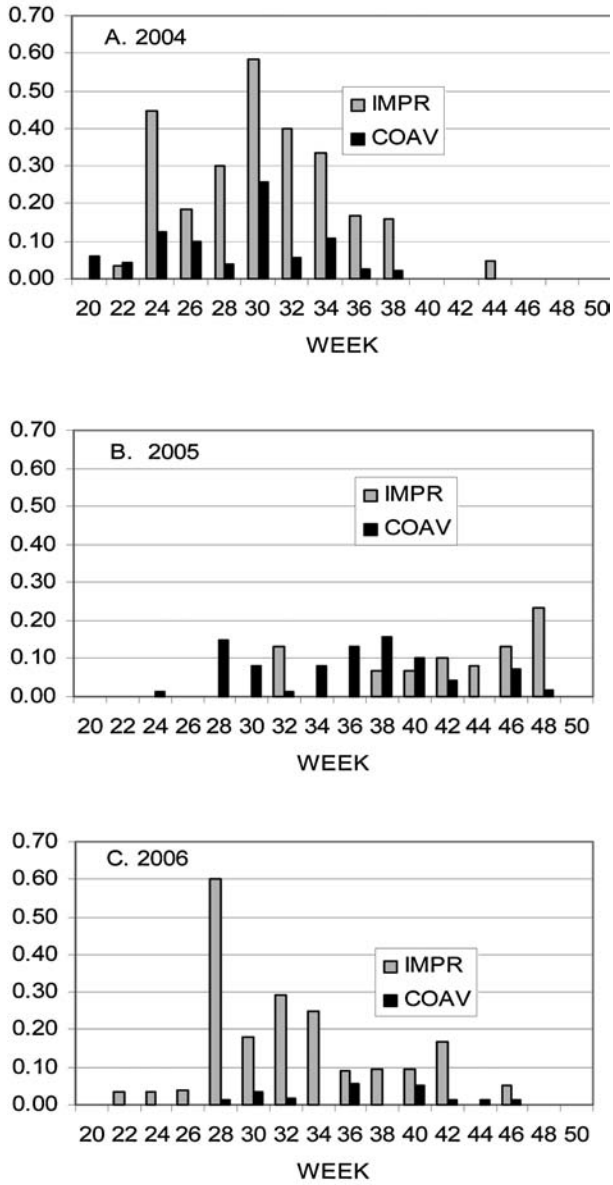

FIG. 12. Seroconversion rates in sentinel chickens (proportion of negative chickens seroconverting per 2-week interval) deployed in Coachella Valley and Imperial Valley along the southern shore of the Salton Sea plotted as a function of disease weeks during 2004–2006 (for flock locations, see Fig. 2).

tion hunting along ecotonal vegetation at North Shore. It is also important to note that these treatments commenced almost one month after the initial detection of WNV activity at North Shore, and although not detected by our surveillance program, virus had apparently broken out of the North Shore target area.

In 2005, virus was not detected until 29 May, more than 1 month later than in 2004. Most likely, the virus was active prior to this date of initial detection and had already dispersed throughout the Coachella Valley. In agreement, WNV was detected in the upper valley immediately after detection at North Shore, precluding any attempt at containing dispersal. Enzootic surveillance in the upper valley was enhanced during 2005 by extensive use of gravid female traps, and their use contributed to the increased infection rates recorded in Table 1. Five human cases were reported during 2005, similar to the seven reported during 2004.

Timely aerial ULV treatments at North Shore in 2006 appeared to interrupt the early season amplification, contained early dispersal of WNV out of the North Shore area, limited the involvement of *Cx. quinquefasciatus*, and may have prevented tangential transmission to humans. Factors contributing to the success of the aerial treatments included the ability of the aircraft to reach large acreage not accessed by road, especially the shoreline vegetation, treatment of large acreage ahead of the dispersal track of the virus, and repeated 26 treatments, weather permitting, over a period of 40 nights. Although the calculated percent control averaged only 61%, repeated treatments apparently compensated for gaps in coverage and missed targets. Surveillance data from our comparison areas in the Imperial Valley that did not receive adulticide treatments indicated that WNV activity was greater during 2006 than in previous years, in marked contrast to the decrease reported in the Coachella Valley. Because the two valleys are close in proximity and similar in climate and seasonal mosquito-borne virus activity (Reisen et al. 1996), we feel that these results support our contention that intensive early season aerial treatments during 2006 were successful in delaying the amplification of virus in the rural lower valley and thereby prevented virus dispersal to the upper valley. An understanding of the landscape ecology of arboviruses in Coachella Valley provided us with an early season target for focused intervention that resulted in a long term and widespread impact upon virus amplification during the en-

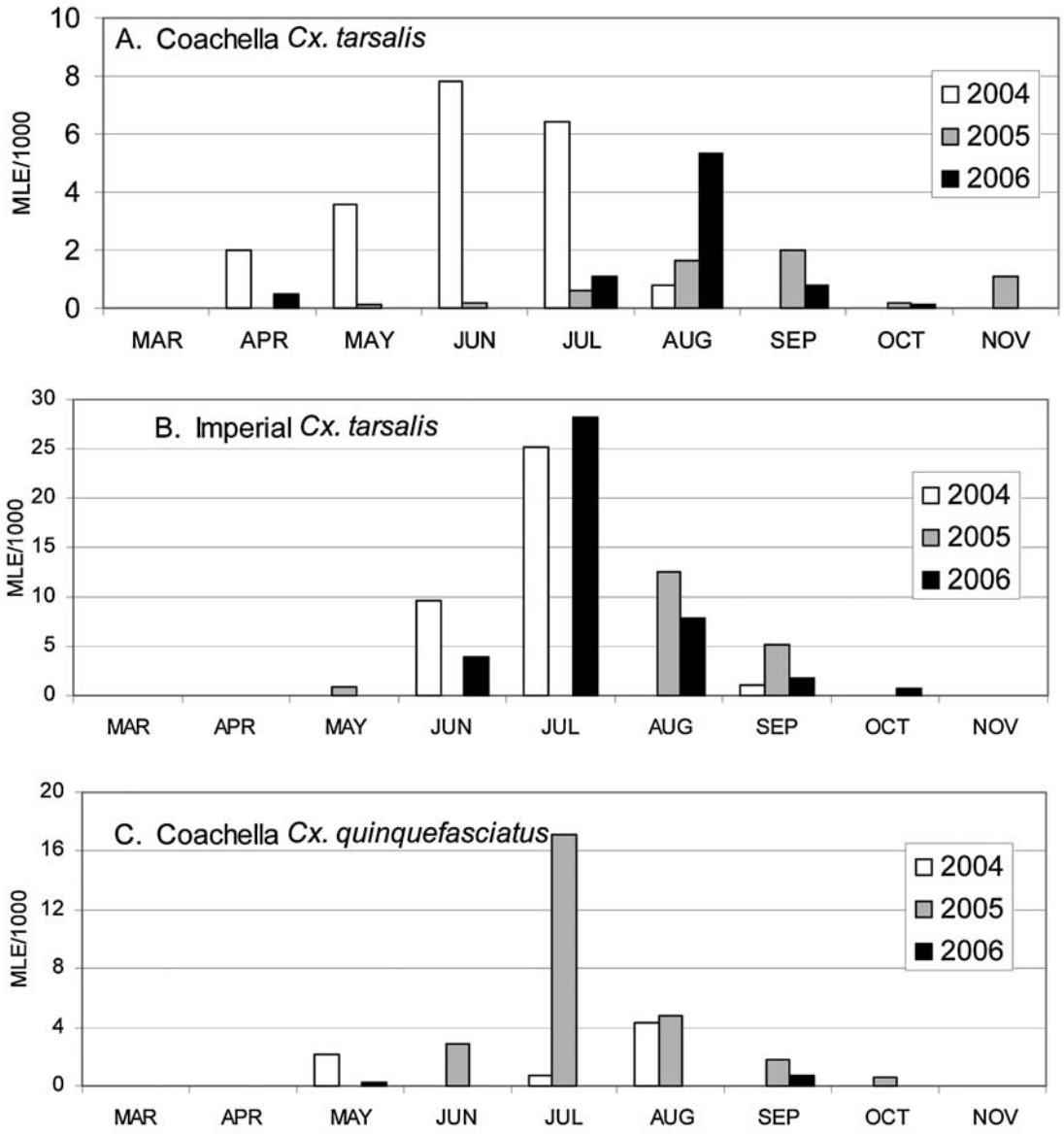

FIG. 13. Maximum likelihood estimates of West Nile virus (WNV) infection per 1,000 females tested plotted by month for populations of *Cx. tarsalis* in Coachella and Imperial valleys and *Cx. quinquefasciatus* in Coachella Valley, 2004–2006.

suing season. These unique data show the value of long term ecological studies and early season intervention.

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DISCLOSURE STATEMENT

No conflicting financial interests exist.

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Culicoides midge trap enhancement with animal odour baits in Scotland

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Abstract. Examples of the commercial trap Mosquito Magnet® Pro™ (MMP emitting attractant 1-octen-3-ol in carbon dioxide 500 mL/min generated from propane fuel), were run 24 h/day on the Isle of Skye, Scotland, during June–August 2001 and evaluated for catching *Culicoides* biting midges (Diptera: Ceratopogonidae). From 30 days trapping, the catch averaged 2626 ± 1358 *Culicoides* females/trap/day (mean ± SE, range 558 ± 139 to 6088 ± 3597, for five sets of six consecutive nights), predominantly the pest *Culicoides impunctatus* Goetghebuer (68% overall), plus *C. vexans* (Stæger) > *C. delta* Edwards > *C. pulicaris* (L.) > *C. lupicaris* Downs & Kettle > *C. albicans* (Winnertz) > other *Culicoides* spp.

Attempts were made to enhance the odour baiting system by adding hexane-extracts (2.1 mg/day) of hair samples from large host animals, resulting in the following effects on *Culicoides* collections: sheep −53%, red deer −26%, calf +20%, pony +40%, water buffalo +262%, with greatest increases for *C. impunctatus and C. pulicaris.*

Serial concentrations of these animal extracts ($10^{-1} - 10^{-3} \times 2.2$ g/mL) were assayed on parous female *C. impunctatus* response in a Y-tube olfactometer (airflow 150 mL/min), and by electroantennogram (EAG) on *Culicoides nubeculosus* Meigen laboratory-reared parous females. Positive behavioural responses to host odours were dose-dependent: the water buffalo extract being most active (threshold 0.22 g/mL), similar to deer, whereas other host extracts were ≥ 10-fold less active. Correspondingly, the EAG threshold was lowest for water buffalo, 10-fold greater for deer, calf and pony, but not detected for sheep. If the active component(s) of these host extracts can be identified and synthesized, they might be employed to improve the capture of *Culicoides* midges for local control by removal trapping.

Key words. *Culicoides impunctatus*, *C. nubeculosus*, *C. pulicaris*, *C. vexans*, animal odours, attractants, baited traps, biting midges, carbon dioxide, electroantennogram, midge control, Mosquito Magnet®, octenol, odour baits, removal trapping, Scotland.

Introduction

Biting midges of the genus *Culicoides* cause severe disruption to outdoor industry and recreation in many parts of the World. Seasonally in Scotland, *Culicoides* females become major pests of people and livestock, particular in the Scottish Highlands and West Coast areas. At least 37 species of *Culicoides* occur in Scotland (Boorman, 1986), having a negative impact on the local economy through their biting attacks on man, affecting rural industry, particularly forestry (Hendry & Godwin, 1988) and tourism (Blackwell, 2001; Blackwell & Page, 2003). *Culicoides* biting pressure forces red deer herds off low-lying pastures onto higher, nutritionally poor slopes (Hendry, 2000) and may cause

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distress in cattle, reducing milk yields in Highland areas (Cameron, 1946). Horses suffer allergic reactions to midge bites, including acute dermatitis known as 'sweet itch'.

Among the biting midge fauna of Scotland, *Culicoides impunctatus* outnumbers all other species: its legendary attacks having serious consequences for human wellbeing and industry (Hendry & Godwin, 1988; Hendry, 2000; Blackwell, 2001). This species breeds abundantly in the damp, acidic soil characteristic of the Scottish Highlands (Blackwell *et al.*, 1999). Females of *C. impunctatus* are primarily autogenous, requiring a bloodmeal after completion of their first gonotrophic cycle and oviposition, in order to produce another batch of eggs (Blackwell *et al.*, 1992). Their preferred hosts are various ungulate mammals (e.g. cattle, deer and horses), with humans bitten only opportunistically (Blackwell *et al.*, 1994a, 1995).

Like other arthropods, *Culicoides* females employ semiochemicals for host-location (Mordue, 2003). For *C. impunctatus*, host-related kairomones and an aggregation pheromone have been identified, the latter having an additive effect with one of the key host kairomones, 1-octen-3-ol (Blackwell *et al.*, 1994b, 1996; Bhasin *et al.*, 2000a). Additional host-related kairomones for *C. impunctatus* include acetone, butanone and L (+) lactic acid (Bhasin *et al.*, 2000a,b, 2001); presumably these olfactory stimuli complement visual cues for midge attraction to a host. Also carbon dioxide, commonly involved in the activation and orientation of haematophagous insects, enhances the response of *C. impunctatus* females to black targets simulating host animals (Bhasin, 1996).

Currently there is no effective means of controlling *Culicoides* biting midges in Scotland: it is impractical to apply insecticides or undertake habitat manipulation on sufficient scale (Kettle, 1962). Personal protection by topical application of insect repellent requires frequent re-treatment; such products vary in their effectiveness and concerns have been raised about safety of DEET (Selim *et al.*, 1995; Wantke *et al.*, 1996; Hayley & Kurt, 1997), the active ingredient of most readily available brands of insect repellents.

Considering the widespread abundance of biting midges in Scotland and many other parts of the world (Kettle, 1977), the control strategy should aim to limit their numbers attacking people and livestock in key localized areas. To avoid using pesticides against mosquitoes, various types of 'removal traps' are being commercially marketed, based on their power to collect mosquitoes rather than proof of population control (Day & Sjogren, 1994; Kline, 2002): mostly based on the attractant 1-octen-3-ol carried by carbon dioxide (produced by various means), with killing mechanisms including electrocution, drowning, sticky boards, live-catching and desiccation. For example, several models of the Mosquito Magnet® (http://www.mosquitomagnet.com, American Biophysics Corporation, East Greenwich, RI, U.S.A.) have been evaluated for trapping and surveillance of mosquitoes (Kline, 2002; May *et al.*, 2002; Ritchie *et al.*, 2003; Turell *et al.*, 2003) and *Culicoides* spp. in Florida (Cilek *et al.*, 2003). To gain further experience with this device, we investigated the Mosquito Magnet® Pro™ (MMP) for trapping *Culicoides* midges in Scotland, and assessed additional odours that might enhance the MMP trap specificity for the notorious Scottish midge *C. impunctatus*.

Materials and methods

Attractant modification: animal extracts

For attempts to modify the standard MMP attractant 1-octen-3-ol, samples of hair were taken from individual red deer (*Cervus elaphus* Linnæus), sheep (*Ovis aries* Linnæus), calf (*Bos taurus* Linnæus), water buffalo (*Bubalus bubalis* Linnæus) and pony (*Equs cabellus* Linnæus). From each host type, 22 g of hair was soaked in 20 mL hexane (> 99%: Sigma-Aldrich, Gillingham, Dorset, U.K.) for 7 days at 5°C. Samples were filtered through cotton wool and concentrated to 50%, under nitrogen, giving elution concentration from 2.2 g hair per mL hexane. Dilutions of these animal hair extracts were used for laboratory tests and field trials.

Electrophysiological assays

Using our local strain of *Culicoides nubeculosus*, maintained in the laboratory by the methods of Boorman (1974), electroantennogram (EAG) observations were undertaken to provide preliminary information on antennal sensitivity to the different host extract odours and repellents (Blackwell *et al.*, 2004). This model species was considered acceptable, since a previous comparison of EAG profiles and behavioural assays between laboratory-reared *C. nubeculosus* and field-collected *C. impunctatus* indicated that the same kairomones are utilized by both species (reflecting their similar blood-feeding habits), with *C. nubeculosus* being slightly less sensitive than *C. impunctatus* (Bhasin *et al.*, 2000a).

EAGs were recorded from antennae of unfed *C. nubeculosus* parous females, following procedures developed previously (Blackwell *et al.*, 1994b, 1996, 1997), using the excised head mounted between silver wire electrodes encased in glass microcapillaries (GC 150–10: Clark Electromedical Instruments, Edenbridge, Kent, U.K.) filled with Beadle–Ephrussi Ringer solution (Ephrussi & Beadle, 1936). By means of a four-channel IDAC data acquisition controller (http://www.idac.co.uk), signals were interfaced to a computer and recorded with 'EAG 2000' software (Syntech, Hilversum, the Netherlands). Each test animal extract solution (10 μL of each dilution: $10^{-1} - 10^{-3} \times 2.2$ g/mL hexane) was dispensed onto a filter paper strip in a glass Pasteur pipette and pulsed for 0.5 s into a constant stream of charcoal-filtered humidified air (1 L/min) directed at the antenna. Responses were corrected for antennal fatigue with a standard stimulus of 0.0010% citronellol (85% purity: Sigma-Aldrich, Gillingham,

Dorset, U.K.) presented before and after each experimental stimulus, allowing the calculation of corrected EAG values (Blackwell *et al.*, 1993).

Behavioural bioassays

The responses of freshly netted, parous unfed female *C. impunctatus* to test odours (i.e. animal solvent extracts) were assessed in a horizontal glass Y-tube olfactometer, as described previously (Blackwell *et al.*, 1994b). Test odours (10 μL on filter paper squares) were held in glass conical flasks, through which charcoal-filtered, humidified air was drawn into the two arms of the Y-tube, with a regulated flow rate of 150 mL/min through each arm (Platon Flow Control Ltd, Bramley, Hampshire, U.K.), produced by a diaphragm vacuum pump (Vak +1, KNF Neuberger, Witney, Oxfordshire, U.K.). Groups of ~30 female *C. impunctatus* were tested against each of the test odour dilutions: $10^{-1} - 10^{-3} \times 2.2$ g/mL. Insects were allowed 3 min to react, after which the numbers that had moved into either arm or which had not reacted (i.e. remaining at any point in the stem of the Y-tube) were recorded. There were 20 trials per test odour concentration (connected to the left-arm for 10 trials and to the right-arm for 10 trials) with a solvent-only control connected to the other arm of the Y-tube. Test and control papers were renewed every two trials. To establish the expected distribution of *Culicoides* in the Y-tube, 10 'control vs. control' trials were run before each test concentration was investigated.

Field trapping

Two MMP traps were operated during June–August 2001 at an established field site (57°14′ N, 05°14′ W, 50 m a.s.l., Grid Ref. NG660230, OS Landranger 32) on the Isle of Skye, Scotland. The propane-powered MMP trap uses 'counterflow technology™' involving two concentric columns of air moving in opposite directions. The inner column of air has CO_2 added at 500 mL/min and is heated as it passes over a cartridge releasing 1-octen-3-ol at a rate of 6–8 mg/h, then pumped out to disperse the attractants away from the trap. The outer column of air acts to pull flying insects into a fine mesh polyester bag.

From each test animal extract, 1 mL was placed in a slow release vial (giving a release rate of 2.1 mg/day) and attached to the attractant carrier of one of the traps. The two traps were placed side by side, the other serving as the control. After 3 days the test odour was removed and a fresh sample was placed in the second trap, to counteract any trap bias. Trap nets were emptied daily and *Culicoides* females were identified to species, using the key by Campbell & Pelham-Clinton (1960), and counted. At the start of each 6-day trial of each animal extract, the cartridge (containing 1660 mg 1-octen-3-ol) was replaced in both traps. On the night before the 6-night series testing each extract, to check that there was no directional preference for either of the traps, both traps were run side-by-side for one night without additional attractant sources.

Statistical analysis

EAG recordings (mV) were corrected for antennal fatigue using the following equation: $C = (2 \times E/S_b + S_a) \times S_i$, where C = corrected EAG value, E = experimental value, S_b = standard before the test compound, S_a = standard after the test compound, and S_i = initial standard (Blackwell *et al.*, 1993). Electrophysiological thresholds (i.e. where the test response became significantly greater than the control value) were assessed by comparing control and corrected values (two-sample *t*-tests). Differences between the corrected absolute EAGs for each extract were assessed using one-way analysis of variance (ANOVA) followed by Fisher's LSD procedure.

Olfactometer data were analysed by chi-squared (χ^2) tests, having established no significant differences between a 50 : 50 distribution in the 'control vs. control' trials.

Culicoides impunctatus catches in the test MMP traps were converted to percentages of the total trap catches (i.e. control + test). Differences between the control and test catches were assessed by two-sample *t*-tests on arcsine √-transformed data.

Results

Electrophysiological assays

EAG dose–response curves were obtained from female *C. nubeculosus* antennae exposed to one or other of the five animal extracts (Fig. 1), with electrophysiological thresholds recorded at the basic concentration (i.e. extract of 2.2 g/mL) for deer, calf and pony extracts. For sheep extract, no EAG threshold was attained, whereas for water buffalo the EAG threshold was 0.22 g/mL.

Behavioural bioassays

Over the full range of experimental concentrations (i.e. $10^{-1} - 10^{-3} \times$ extract of 2.2 g/mL), each extract was 'attractive' (measured as the movement of midges into the arm of the Y-tube carrying the test odour) to parous female *C. impunctatus*, although this attraction was not statistically significant (marked * in Fig. 2) until the higher end of the dilution series was reached: 0.22 g/mL for red deer and water buffalo extracts; 2.2 g/mL for sheep, pony and calf extracts. For all host extracts, attractancy to *C. impunctatus* was dose-related but not directly proportional (Fig. 2).

Field trapping

From 30 days operation of standard MMP traps, the catch averaged 2626 ± 1358 *Culicoides* females/trap/day

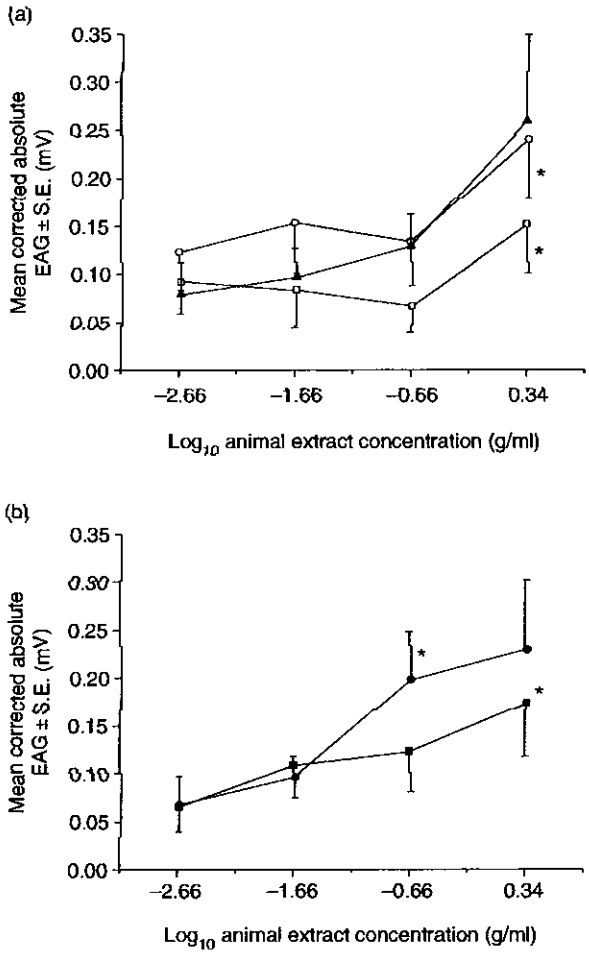

Fig. 1. Mean corrected absolute EAG responses from unfed, parous female *C. nubeculosus* antennae in response to dilutions of (a) calf (□), deer (○) and sheep (▲) extract and (b) pony (■) and water buffalo (●) extract (all extracts prepared in hexane). *Response threshold (i.e. significantly greater EAG response than solvent only control response): calf, 2.2 g/mL, $t = 2.37$, $P = 0.032$; deer, 2.2 g/mL, $t = 2.14$, $P = 0.05$; pony, 2.2 g/mL, $t = 2.28$, $P = 0.043$; water buffalo, 0.22 g/mL $t = 2.23$, $P = 0.046$. $n = 10$ per compound. Vertical bar = standard error.

(mean ± SE, range 558 ± 139 to 6088 ± 3597, for five sets of six consecutive nights). The dominant species of biting midge collected in MMP traps throughout the season on the Isle of Skye was *C. impunctatus*, comprising ≥ 68% of the overall catch. Of the remaining species caught in good numbers, *C. delta* and *C. vexans* were prominent at the beginning of the season. The proportion of *C. pulicaris* increased from ~5% in June to 10–18% in July–August and the proportion of *C. lupicaris* also rose in August. *Culicoides albicans* was relatively scarce (0–7%) until the final trial (24 August onwards), when this species represented 20.7% of the total catch.

The relative proportions of *Culicoides* caught in paired MMP traps with and without emission of the host extract odour varied according to the animal type (Table 1). As the different animal extracts were investigated at different times during the season, with natural variation in proportions of species comprising the *Culicoides* flock, results from each host-test series were not directly comparable. Of the different host odours (released at 2.1 mg extract/day), only water buffalo odour increased the *Culicoides* catch significantly, with a mean of 79.6 ± 4.5% of the total catch. Calf extract was also associated with higher catches in the odour-baited MMP trap compared to the trap without host extract, although this difference did not attain statistical significance. When neither of the paired MMP traps emitted host extract odour, the *Culicoides* averaged 44.6% ('left' trap) and 55.4% ('right' trap) (Fig. 3).

To see if the host species extract affected the proportions of *Culicoides* species collected, we compared the species composition in traps baited with or without extract of water buffalo, the most productive host odour (Fig. 3). This revealed that the greater collection was mainly due to significantly increased numbers of *C. impunctatus* and *C. pulicaris* (Fig. 4).

Discussion

By recording EAG responses to certain host odours (obtained from hair extracts), this investigation augments the limited information from the few previous studies applying electrophysiological recording techniques to *Culicoides* antennae. Hitherto, midge EAGs were used to assess known repellents, including several isothiocyanates (Blackwell *et al.*, 1997) and the oil of bog myrtle, *Myrica gale* L. (Myricaceae), a common Highland shrub of potential utility for extraction of a natural repellent for use against biting flies (Evans *et al.*, 1996), and attractants likely to be involved in host location (Blackwell *et al.*, 1994b, 1996; Bhasin *et al.*, 2000a). In the present study, EAG thresholds were determined for extracts from four types of host (calf, deer, pony and lowest for water buffalo), but not for sheep, which may be naturally less attractive to biting midges (see below).

EAG dose–response curves generally have an initial steep log-phase, followed by a plateau phase at higher concentrations, representing saturation of odour receptors specific for the test compound, perhaps in addition to indiscriminate stimulation of other classes of receptor. Among EAG responses of *C. nubeculosus* to the animal extracts tested, only the curve for water buffalo extract showed any degree of plateauing out, with the remainder apparently still in their log-phase at the highest concentration of the respective extracts. This may have been due to the active component chemicals being in relatively low concentrations. The initial extracts were concentrated under nitrogen to give a hair weight:volume ratio of 1 g:450 μL. By comparison, the only other study that has recorded *Culicoides* EAGs in

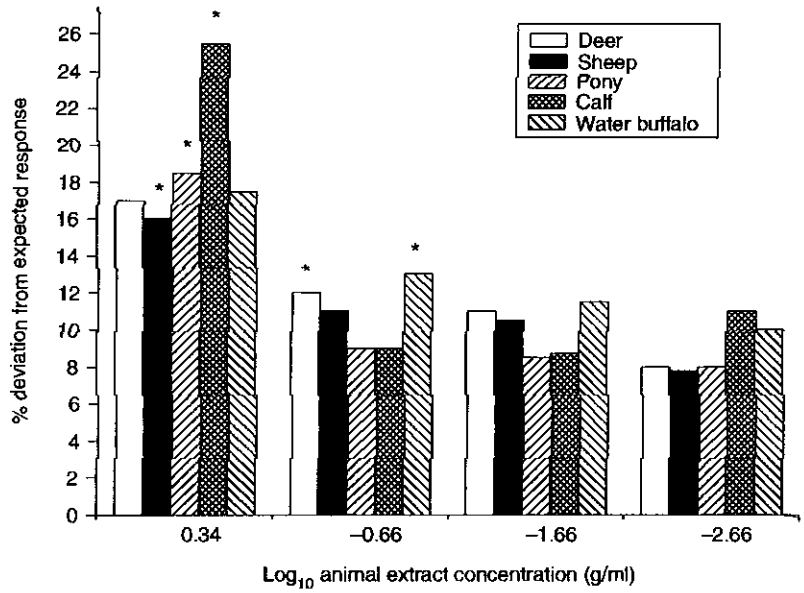

Fig. 2. Response of freshly collected, parous female *C. impunctatus* in a Y-tube olfactometer when offered the choice between odour from a dilution series of each test animal extract or a control odour (solvent only) to move towards. Data are shown as the percentage deviation from the expected response. *Threshold value, i.e. where the response became significantly different from the control response, as shown by the percentage deviation from the expected response following χ^2 analysis (deer: $\chi^2 = 4.36$, $P < 0.05$; sheep: $\chi^2 = 5.05$, $P < 0.05$; pony: $\chi^2 = 9.49$, $P < 0.01$; calf: $\chi^2 = 14.73$, $P < 0.001$; water buffalo: $\chi^2 = 4.61$, $P < 0.05$).

response to animal extracts (Bhasin, 1996) had a final weight : volume ratio of 1 g : 500 µL.

Of the test animal extracts, the order of electrophysiological activity with female *C. nubeculosus* (from the EAG thresholds) was: water buffalo > calf ≡ red deer ≡ pony > sheep. Comparing these data with a previous study recording the response of female *C. impunctatus* to whole and partitioned extracts of horse, deer and cow (with cow giving the largest EAG responses), confirms previous suggestions that *C. impunctatus* and *C. nubeculosus* have similar antennal receptor profiles (Bhasin *et al.*, 2000a). However, caution is required in making this suggestion, as, in the previous study, only one concentration of each extract was presented to the antennae, rather than a dilution series as in the present study. It is difficult therefore to calculate differences in antennal sensitivity between the two species, particularly as the exact chemical composition of the different extracts was unknown. Further evidence that *C. nubeculosus* and *C. impunctatus* have similar behavioural and electrophysiological responses comes from *C. nubeculosus* lack of EAG response to sheep extract and *C. impunctatus* not responding behaviourally to sheep extract in the olfactometer. Moreover, *C. impunctatus* seldom takes bloodmeals from sheep, but feeds mostly on other large mammals in the field (Blackwell *et al.*, 1994a, 1995).

All five host extracts were attractive to *C. impunctatus*, with responses to sheep, pony and calf becoming statistically significant at the highest concentration (extract of 2.2 g/mL), whereas the deer and water buffalo extracts were significantly attractive at 10-fold lower concentration (extract of 0.22 g/mL), agreeing with the field preference for deer and cattle as a source of bloodmeals for this species (Blackwell *et al.*, 1994a, 1995). Similarly, Bhasin (1996) found attraction of *C. impunctatus* to horse, deer and cow but not sheep, using extracts obtained by a different procedure. Such concordance of results offsets the limitation that

Table 1. Mean ± SE *Culicoides*/trap/day: comparison of MMP traps with standard bait (CO_2 + 1-octen-3-ol) or with standard bait plus animal extract odour released at 2.1 mg/day ($n = 6$ tests per row).

	Culicoides/trap/day		
Animal extract	Standard bait	Odour bait	Difference
Calf (*Bos taurus*)	558 ± 139	668 ± 193	+20%
Water buffalo (*Bubalus bubalis*)	1515 ± 746	5488 ± 2777	+262%
Red deer (Cervus elaphus)	*2282 ± 1218*	*1683 ± 412*	*−26%*
Sheep (*Ovis aries*)	2626 ± 1088	1243 ± 641	−53%
Pony (*Equs cabellus*)	6088 ± 3597	8510 ± 3462	+40%

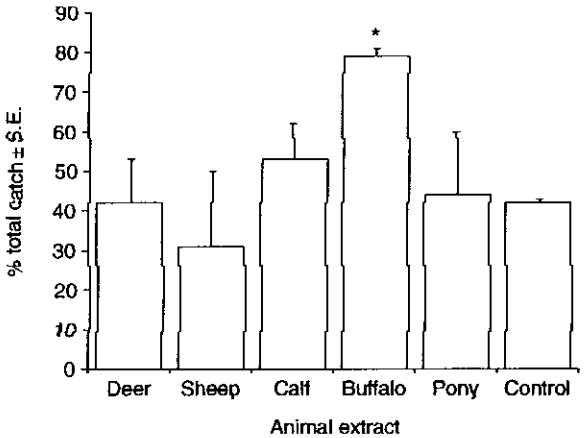

Fig. 3. *Culicoides* females in MMP traps baited with test odours (animal extracts, released at 2.1 mg/day), as percentage of the total catch in both control and experimental traps ($n = 6$), compared with the control (two standard MMP baited traps, i.e. CO_2 + 1-octen-3-ol). *Significantly different from control catch ($t = 6.63$, $P = 0.001$).

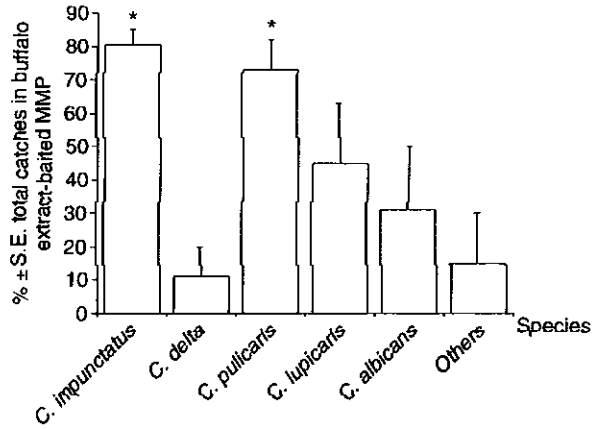

Fig. 4. *Culicoides* females in MMP traps baited with water buffalo extract (2.1 mg/day), as percentage of the total catch in both control and experimental traps ($n = 6$). *Significantly different from control catch (*C. impunctatus*: $t = 9.86$, $P < 0.001$; *C. pulicaris*: $t = 4.08$, $P = 0.003$).

neither Bhasin (1996) nor the present study used multiple examples of each host species.

Among the five host extracts added to MMP emissions in the field trials, only water buffalo significantly enhanced the trap catches of both *C. impunctatus* and *C. pulicaris* (overall 2.6-fold increase). Extracts of pony (40% increase) and calf (20% increase) also yielded greater MMP trap collections of *Culicoides*, but these were not significantly more. In a comparable study, Bhasin (1996) detected no significant differences between catches in odour-baited and unbaited Delta traps that collect midges in 'tens', whereas the Mosquito Magnet® catches tens of thousands – making the contrasts more obvious.

Apart from the exotic water buffalo, the failure of other host extracts to augment the yields of *Culicoides* in MMP traps could be explained by the low dose (2.1 mg/day) or the masking effect of 1-octen-3-ol (6–8 mg/h). It has been shown previously that, as the concentration of 1-octen-3-ol rises, it appears to induce receptor saturation (Bhasin *et al.*, 2000a). Hence, it would be more revealing to evaluate a range of 1-octen-3-ol bait concentrations combined with other host odours, in an attempt to optimize their combined effectiveness as attractants. Also it would be more convenient to replace the animal extract with purified and/or synthetic active components: investigations are underway to determine the cocktail of chemicals that were responsible for the observed field attractancy of the water buffalo extract. Thus, we are optimistic that better baiting systems can be devised for *Culicoides* traps, perhaps combining the water buffalo attractant(s) with those from cow urine detected by Bhasin *et al.* (2001), particularly for localized removal trapping of the Scottish midge pest *C. impunctatus*.

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GUIDELINES FOR "OPEN MARSH WATER MANAGEMENT" IN DELAWARE'S SALT MARSHES - OBJECTIVES, SYSTEM DESIGNS, AND INSTALLATION PROCEDURES

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Abstract. Open Marsh Water Management (OMWM) is a method for controlling salt-marsh mosquitoes using physical alterations of marsh habitat. Ponds and ditches are selectively excavated in order to create unsuitable environs for mosquito eggs and larvae while creating suitable habitat for larvivorous fishes. Based on environmental effects observed at two experimental sites, plus operational experiences in Delaware and adjacent states, guidelines are presented for designing and installing OMWM systems in Delaware. These guidelines should be applicable to other salt marshes from New England to Florida that have similar environmental characteristics.

The guidelines are intended to produce OMWM systems that will control mosquitoes while minimizing long-term ecological disruptions of the marsh community. They emphasize the following topics, including the environmental or economic reasons for why particular approaches were chosen: 1) use and location of open tidal ditches; 2) interspersion of open tidal ditches with closed, non-tidal ponds and pond radial ditches; 3) use of semi-tidal sill ditches and ponds; 4) incorporation of OMWM systems into previously parallel-grid-ditched marshes; 5) permissible lowering of the water table elevation in relation to local marsh surface, as caused by spoil deposition on the marsh surface and/or drainage from open tidal ditching, but not to such an extent that the original vegetation is replaced by other species during vegetative recovery; 6) protocols for designing, demarcating and installing OMWM systems; 7) density, depth and surface areas of ponds; 8) geometric vs. naturalistic excavations; 9) habitat enhancement for waterfowl use; 10) water quality, fish kills and dependable mosquito control; 11) OMWM alterations under special situations, such as upland border marshes with excessive freshwater runoff or marshes with intensive muskrat burrowing activity; 12) blending of OMWM with other marsh management goals.

INTRODUCTION

Open Marsh Water Management (OMWM) is a method for controlling salt marsh mosquitoes using physical alterations of marsh habitat. OMWM alterations involve selective excavation of ponds and ditches which create unsuitable environs for mosquito egg deposition and larval maturation, while simultaneously providing stable habitats for larvivorous fishes (Ferrigno and Jobbins 1968; Ferrigno *et al*. 1975). As such, OMWM promotes and maximizes biological control through physical manipulations. The Delaware

Mosquito Control Section is proposing to use OMWM, where appropriate, as a primary means of salt-marsh mosquito control on much of Delaware's tidal wetlands. It has become obvious that a set of operational guidelines is necessary for OMWM system design and installation. Since water management practices, when incorrectly conceived or installed, have potential for adverse environmental impacts (Daiber 1982), it is essential that a protocol for design and installation be formulated in order to avoid detrimental effects.

Guidelines for OMWM have been written for New Jersey (Bruder 1980) and Maryland (Lesser 1982). These guidelines adequately define the local OMWM process for regulatory or permitting agencies, but do not fully address the reasons for many recommended procedures, nor do they account for OMWM use under unusual conditions (e.g. in atypical border marshes, in areas of snow goose feeding). Guidelines for OMWM in Massachusetts are being prepared by Hruby and Montgomery (ms. in prep.) which provide greater insights into the "why" of recommended procedures. The Delaware OMWM guidelines attempt to elaborate upon the reasons for recommended procedures. Our guidelines should be applicable to other mid-Atlantic regional marshes, and also to marshes of similar environmental characteristics (e.g. tide range, vegetation, soil type) in New England and along the southeast Atlantic coast. The Delaware OMWM protocol is partially based on the experiences and recommendations of OMWM programs in New Jersey and Maryland; it is strongly influenced by studies done by the Delaware Mosquito Control Section, sponsored by the Delaware Coastal Management Program (DCMP), of the environmental effects of prototype OMWM systems on marshes of the Bombay Hook and Prime Hook National Wildlife Refuges (Meredith et al. 1983); and it also relies on observations of operational OMWM systems in Delaware which were begun in 1980.

OBJECTIVES FOR OMWM IN DELAWARE

The objectives of OMWM in Delaware are as follows:

1) Control of Pestiferous Salt-Marsh Mosquitoes

The primary objective is to provide a water management technique that will control the dominant species of Delaware salt-marsh mosquitoes: *Aedes sollicitans*, *Aedes cantator*, *Aedes taeniorhynchus*, *Culex salinarius*, and *Anopheles bradleyi* (Lake 1973).

2) Reduction in Use of Chemical Insecticides

If the primary objective is achieved, then the current reliance on chemical insecticides will be reduced. Successful control via OMWM will be considered achieved if the frequency of insecticide spraying on a given marsh is at least 80% less after OMWM than before OMWM.

3) Minimize Adverse Secondary Impacts on the Marsh Community

The application of OMWM should not adversely impact other existing marsh resources or functions. A primary gross environmental alteration to be avoided when using OMWM is promotion of higher elevation plants through increasing the marsh surface elevation due to spoil deposition and/or excessive lowering of the water table elevation due to drainage. Since the ecological consequences of altering marsh vegetation patterns are not fully

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understood, it is both responsive and prudent to install OMWM systems that will not grossly alter existing vegetation. With minimal alterations to extant vegetation, other components of the marsh community (e.g. surface invertebrates, edaphic alge, detritus production and export) will not be radically changed.

4) Habitat Enhancement for Waterbirds

As a result of the creation of mosquito-control OMWM ponds, habitat may be created that is also beneficial to waterfowl, shore birds, and wading birds (Meredith et al. 1984). OMWM pond creation may help to mitigate the loss of high marsh ponds that historically were abundant on Delaware's salt marshes, but were drained by the old mosquito control method of parallel-grid ditching.

5) Cost-Effective Mosquito Control

The use of OMWM is potentially more cost effective than the use of insecticides. According to economic analyses conducted in New Jersey (Hansen et al. 1976; Shisler et al. 1979; Shisler and Schulze 1985), properly installed OMWM systems will be less expensive than continual treatment with chemical insecticides.

PROTOCOLS FOR OMWM IMPLEMENTATION

1) Marsh Breeding Habitats Where OMWM Could Be Used

The environmental requirements necessary for breeding of salt-marsh mosquitoes are usually delineated by vegetation zones. In Delaware, the most severe breeding habitats are on the highest marshes (i.e. marshland that is only flooded by spring or storm tides, and which often goes dry between rainfalls or surface inundations). Plant species characteristically associated with the high marsh are the salt hay grasses, *Spartina patens* and *Distichlis spicata*, and the short-form of the cordgrass, *Spartina alterniflora*. Short-form *S. alterniflora* in the high marsh may be found in extensive stands, or may be confined to shallow depressions surrounded by salt hay that hold water long enough for a mosquito brood to progress to adult emergence; in either case, zones of short-form *S. alterniflora* can produce severe broods, but usually not at the frequency of salt hay habitat. The salt hay contains two types of breeding sites: 1) discrete, relatively deep potholes; 2) "tussocky" areas that hold surface water at the base of grass clumps. Both of these salt hay sites are major problem habitats. Mosquito breeding can also occur near the upland fringe in salt hay zones which are in association with marsh elder (*Iva frutescens*), groundselbush (*Baccharis halimifolia*), marsh hibiscus (*Hibiscus* spp.), marsh mallow (*Kosteletzkya virginica*), or panic grasses (*Panicum* spp.). Depending upon locality within the State, the short-form *S. alterniflora* found in shallow, mosquito-breeding depressions surrounded by salt hay may be replaced by three-squares (*Scirpus* spp.) or black needlerush (*Juncus roemerianus*), both which can form mosquito-producing habitat under such conditions. A final type of salt-marsh mosquito breeding habitat in Delaware can be found in potholes or depressions in zones of common reed, *Phragmites australis*.

Any of the above described breeding habitats are candidates for OMWM treatment. These marsh breeding habitats are found in extensive, open salt marshes extending landward from tidal rivers and coastal embayments; in

small pocket or finger marshes along the upland fringe; in the more brackish marshes near headwaters of tidal creeks; and in swales behind coastal dunes.

OMWM will not be used in marshes or marsh zones subject to an average of at least one high tide per day, since such areas usually do not produce mosquitoes. Non-breeding marshes or marsh zones in Delaware are typically vegetated by tall- or intermediate-form S. alterniflora. Also, extensive stands of cattails (Typha) spp. or three-squares (Scirpus spp.) are not candidates for OMWM. Permanent ponds on the marsh surface (which are relatively large and deep) do not serve as breeding habitat and will not be drained.

2) Factors Considered in OMWM Site Selection and System Design

OMWM alterations must directly affect potential mosquito breeding sites within known breeding marshes. The determination of which marshes breed, and are thus candidates for OMWM, will be based on historical aerial spray records and/or historical larval inspection records for specific marshes. Potential breeding sites within a candidate OMWM marsh will be identified by staff biologists and/or mosquito control supervisors via on-site evaluations of: 1) vegetative cover, 2) tidal flooding and runoff patterns, 3) physical characteristics of surface depressions, 4) potential for access and survival of larvivorous fishes, and 5) when practical, direct observation and quantification of mosquito larvae. To aid in design of the OMWM systems, other environmental factors may be considered on a site-specific basis. Such factors could include local topographic relief, soil characteristics (particularly peat vs. mineral content), depth of mean water table below local marsh surface, and proximity to critical or unique wildlife habitats. Which factors will be examined for a specific tract, and how they will be integrated to assist in the OMWM system design, will vary from site-to-site. Staff biologists will use this information to aid in formulating regional OMWM design concepts specific to geographic areas.

3) Field Demarcation of OMWM Systems and Alterations

Prior to any excavations, all breeding sites and their specific methods of OMWM treatment will be demarcated with surface stakes. If there is to be an on-site, regulatory review of the proposed alterations, the post-staking stage is the most logical point to have such a review. It is important that a uniform, consistent system for indicating alterations be designed and used by all parties. Since the field lay-out of OMWM systems will be done under supervision of staff biologists and/or mosquito control supervisors, but the actual machine excavations supervised by foremen or machine operators, it is mandatory that excavation personnel be able to interpret and understand the staked designs.

The staking system should clearly indicate pond borders; island locations; location of deeper reservoirs in ponds; the beginning and termination of primary ditches, pond radial ditches, and lateral spurs; where a semi-tidal ditch's shallow sill outlet begins and terminates; and whether or not a ditch approaching a tidal source will be connected at full depth to daily tidal flow. Stake tips can be color coded to indicate various features, and various combinations of stakes used to further discriminate features.

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The staked OMWM design should be drawn on a map which also indicates major natural features. Such maps will be used by the equipment operators and could also be valuable to regulatory agencies. The maps could be of particular value in indicating to which side(s) of an excavation spoil should be directed or placed. This is especially important if spoil is to fill breeding depressions that are not staked for ditching. The amount of excavation planned will be the minimum required to satisfy the OMWM objectives.

4) Excavation Equipment Used in OMWM

Whenever feasible, excavations in OMWM are to be made with a rotary excavator (either amphibious or land-limited). The rotary cutting head broadcasts spoil as a crude slurry, thinly covering the marsh surface for distances up to 15 m from an excavation. Other heavy machinery (e.g. dragline, backhoe, front-end loader) can be used in OMWM as long as the OMWM objectives are met, particularly in regard to satisfactory deposition of spoil. Spoil from non-rotary excavations may be used to fill breeding depressions or old ditches, or can be deposited in small mounds and then spread to a depth less than 10 cm over the marsh surface. Care must be taken during spreading and compaction not to pack the overburden of spoil too densely to permit vegetation recovery. Also, the creation of ruts by the machinery during the spreading process should be avoided. The most likely use for non-rotary equipment in OMWM is to excavate ponds in soils with high mineral content.

5) Removal of Shrubs Impeding Spoil Broadcasting

In some instances, it may be necessary to cut down wetland fringe vegetation, especially shrubs (e.g. *Iva*, *Baccharis*), in order to permit unimpeded broadcasting of rotary spoil. Care should be taken to leave at least a 1.5 m wide band of shrubs along the marsh's upland edge. This will help preserve the natural wetland-upland transition.

6) Location and Ranking of Candidate OMWM Marshes

Of Delaware's 34,500 ha of tidal wetlands, about 6000 ha have been identified as severe salt-marsh mosquito-breeding habitat and are thus candidates for OMWM treatment. Marshes to be treated with OMWM are ranked for work priority according to degrees of breeding severity in relation to human population centers, in terms of both nuisance problems and disease potential. This ranking of work areas may then be modified by factors of landowner cooperation, efficient deployment and transport of heavy equipment, and impact on reduction of aerial spraying.

DESCRIPTION OF OMWM ALTERATIONS

1) Terminology and Types of Alterations

Three types of alteration systems are used in Delaware OMWM: 1) Full-depth tidal ditches (45-90 cm deep), with relatively deep tidal outlets (e.g. 75 cm below marsh surface), plus associated lateral spur ditches, creating a system that has daily tidal exchange; 2) Semi-tidal systems consisting of full-depth ditches (e.g. 75 cm) with a shallow tidal outlet or sill (e.g. 10-20 cm deep - see Fig. 1), plus associated lateral spur ditches landward of the shallow outlet, creating a system that has more tidal exchange than if no ditching was done, but not as much as full-depth tidal

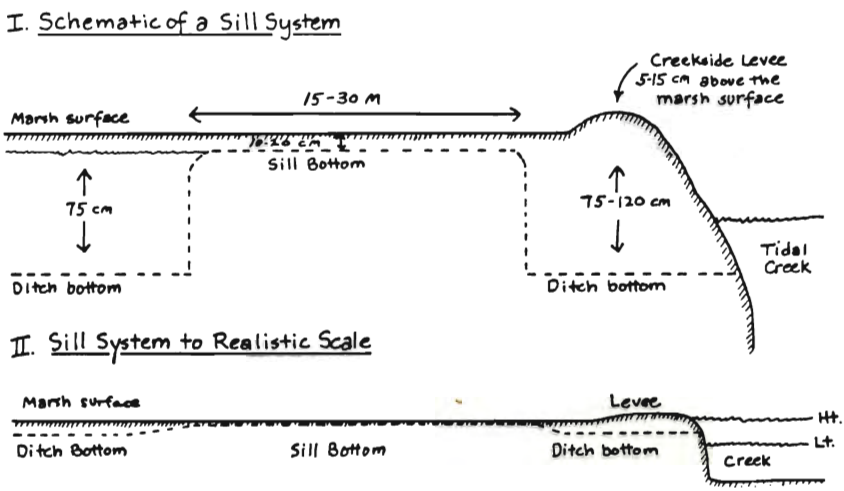

Figure 1. Side-view diagram of a sill (semi-tidal) OMWM system, with emphasis on the sill's shallow outlet.

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ditches; 3) Shallow ponds of 50-1000 square meter surface area (averaging 30 cm deep), with deeper reservoirs (75-90 cm deep), plus associated pond radial ditches of full-depth (e.g. 75 cm), with both ponds and radial ditches lacking any tidal outlets, creating a system that has tidal exchange during only spring or storm tides.

The full-depth tidal ditches with deep outlets are often referred to as "open" systems; when these systems are made semi-tidal via shallow outlets, then these modified systems are known as "sill" systems; the essentially non-tidal systems of ponds and pond radials are often called "closed" systems. The word "Open" in Open Marsh Water Management refers to the fact that OMWM systems do not contain elevated structures above marsh surface to prohibit tidal exchange (e.g. no impoundments, dykes, or sluice gates). OMWM systems may have various combinations of tidal, semi-tidal, and non-tidal systems (i.e. open, sill, and closed systems - see Fig. 2).

When digging open ditches, deeper ditches are preferable since they will not fill-in as rapidly with tidally-borne sediment and will have a longer functional life. Open ditches can be connected to tidal sources at more than one point in order to promote circulation.

The shallow outlets for sill systems should be at least 30 m long in peaty soils and at least 15 m long in mineralogical soils. These lengths will help promote sill longevity in areas where the sill might erode to deeper depths, since the maximum rates of erosion occur at the sill ends. Past the tidal end of the sill, the outlet should slope gradually toward the tidal source in order to minimize undercutting by ebbing water. The shallow sill should not go through any creekside levee since it is along the creekside where sedimentation rates are highest, and where sill longevity would be least. An extra wide (e.g. 150 cm), extra deep (e.g. 120 cm) ditch is constructed at the seaward end of the shallow sill, cutting through the creekside levee. This larger ditch will serve as a "catch basin" for tidally-transported sediments and debris on flooding tides, prolonging the functional longevity of the more landward sill. A correctly designed and installed sill system will remove very shallow, standing surface water from tussocky mosquito-breeding areas while still maintaining a high subsurface water table at low tides. It will also enhance tidal exchange (since the creekside levee has been broached), promoting good water quality to the benefit of larvivorous fishes. Because of the shallow nature of the sill's outlet, breeding depressions greater than 5 cm deep or more than a few meters away from a sill ditch will not be drained; these deeper or more remote breeding depressions should be directly treated with sill ditch lateral spurs.

Closed ponds are excavated in areas of concentrated breeding depressions. Ponds should have a uniform depth of about 30 cm over most of their surface area. Slate (1978) found this depth to be the average depth of potholes containing widgeongrass (*Ruppia maritima*), a valuable waterfowl food. To insure fish survival during droughts, reservoir ditches from 75-90 cm deep should be dug along one or two sides of the pond. Natural or OMWM ponds with several full-depth pond radial ditches (e.g. 75 cm deep) extending outward from the main pond body may not need ditch reservoirs within the pond. Islands should be left in ponds when feasible to provide

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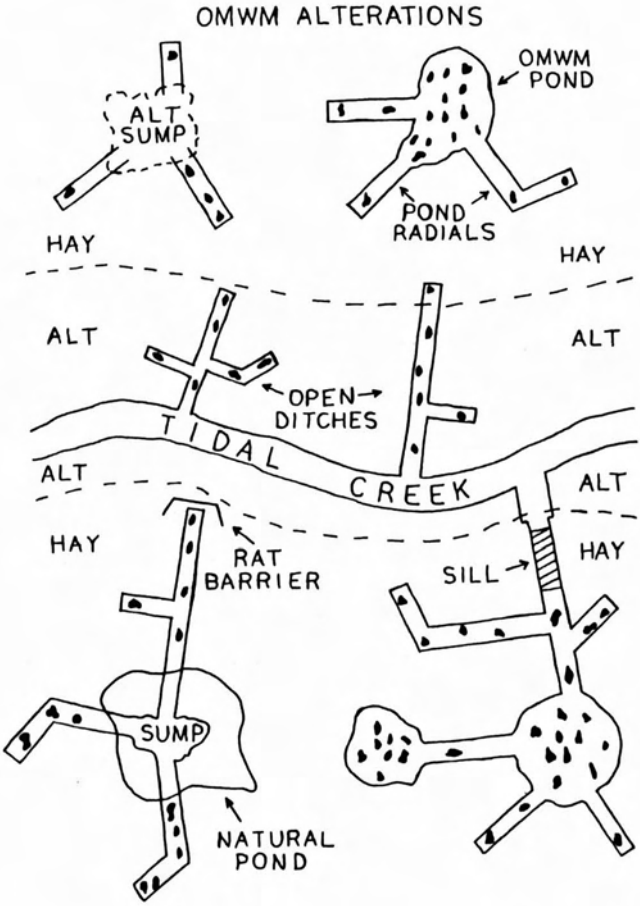

Figure 2. Various marsh excavations and alterations used in the OMWM technique. The darkened spots represent former mosquito-breeding depressions.

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protected areas for bird nesting plus additional edge habitat, while reducing spoil volume around pond perimeters.

2) Interfacing the Old, Parallel-Grid Ditch System with OMWM Alterations

The open tidal ditches of the old parallel-grid ditch network slowly fill with tidally-borne sediment. In the past, these ditches have been cleaned of deposits, restoring the ditch network to its original design and function. This routine cleaning of parallel-grid ditches is a questionable procedure, since many ditches were placed in marshes or sections of marsh that did not require mosquito control, and in some areas these open ditches caused drainage of waterfowl ponds (Clarke et al. 1984) plus excessive depression of the subsurface water table. Routine, wholesale cleaning of the parallel-grid ditch system is not part of Delaware OMWM. Parallel-grid ditches that are filling will not be reexcavated if the cleaned ditches fail to met all of the objectives and specifications for OMWM alterations.

Parallel-grid ditches may be cleaned and restored to open tidal flow in zones of short-form cordgrass where mosquito breeding is evident. Lateral spurs may be dug from these cleaned ditches, treating breeding depressions that might exist between parallel-grid ditches in zones of short-form cordgrass. However, restoring the parallel-grid ditches in salt hay zones to open tidal flow will usually not be done.

Since the installation of sill and closed systems in salt hay areas of the high marsh requires limited or no direct tidal exchange, and since many areas of the high marsh have been treated with parallel-grid ditches, it may be necessary to block, or at least not clean, these high marsh grid ditches. A desirable location for ditch blockage would be at the transition from predominantly short-form cordgrass zones to predominantly salt hay zones. The parallel-grid ditches seaward from this transition edge, in short-form cordgrass zones, could be cleaned and spur ditched if breeding occurs in the lower marsh. Landward from ditch blockages, parallel-grid ditches could be cleaned in order to deepen them for inclusion in sill or closed systems in the high marsh.

If a parallel-grid ditch in the high marsh area has not silted enough to have a short segment of the ditch serve as blockage for a sill or closed system, then spoil "plugs" may be used to achieve blockage. These plugs should fill the parallel-grid ditch to marsh surface level and be at least 8 m long in marshes with mineralogical soil to at least 15 m long in marshes with peaty soils. The plugs should be installed on the salt hay side of a cordgrass-salt hay interface, taking advantage of the more consolidated soils in salt hay zones.

In summation, the basic strategy for managing parallel-grid ditches in Delaware OMWM is to "break-up" the grid network: 1) clean only those ditches that directly contribute to mosquito control; 2) allow other non-breeding ditches to fill naturally; 3) prohibit excessive tidal flow and drainage in the high salt marsh via plugs, thereby restoring standing surface water to the upper marsh with sill and closed systems (see Fig. 3 for an example of OMWM superimposed over a parallel-grid ditch system).

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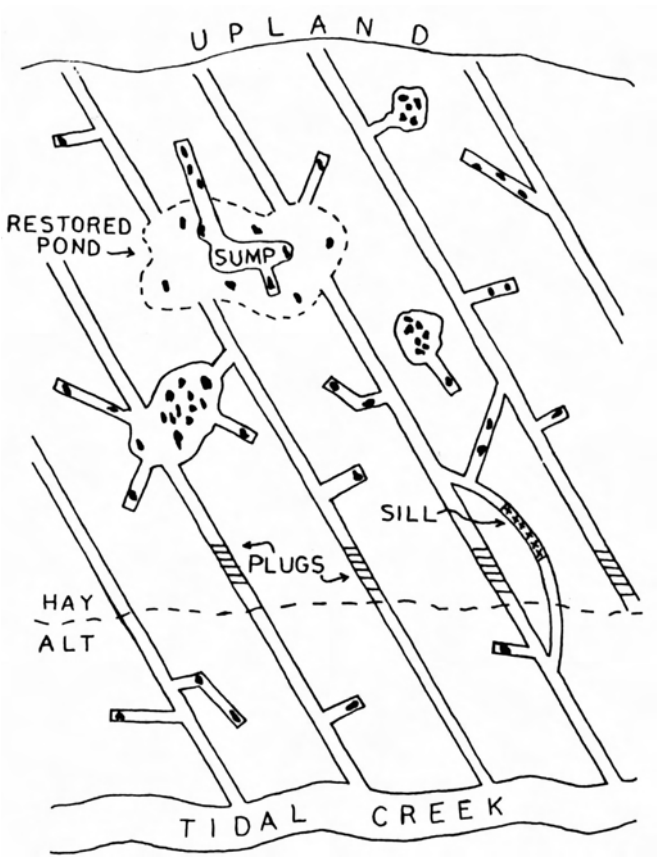

Figure 3. An OMWM system superimposed over a previously parallel-grid ditched marsh. The darkened spots represent former mosquito-breeding depressions.

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TAILORING OMWM ALTERATIONS TO MEET OMWM OBJECTIVES

1) Maintenance of a High Subsurface Water Table

A basic management goal is to insure that no OMWM alteration causes the mean subsurface water table to drop more than 15 cm below local marsh surface elevation. The OMWM studies sponsored by the Delaware Coastal Management Program (DCMP) have found that the mean water table in study site zones of *Iva*, *Baccharis*, and robust *Phragmites* is 15 cm or more below local marsh surface, creating a soil condition that is drier and more aerated than soils in salt hay or short-form cordgrass zones (Meredith *et al*. 1983). In order to discourage conditions that may cause establishment and growth of marsh shrubs and common reed, excessive subsurface drainage and/or excessive spoil deposition, which either separately or in combination may establish a greater than 15 cm average distance between marsh surface and mean water table, should be avoided. While the correlation between vegetation cover type and depth to mean water table may be somewhat variable from site-to-site (especially for *Phragmites*, which may grow in areas of considerable tidal flooding), the avoidance of creating a mean distance between the marsh surface and water table greater than 15 cm provides an initial management criterion for maintaining existing vegetation patterns.

Spoil from ditches and ponds should be spread over the marsh surface at initial depths no greater than 10 cm (after a period for spoil settling, any permanent increase in surface elevation should be less than 5 cm).

Generally, open tidal systems should not be put in areas of salt hay. However, breeding depressions in salt hay within 3 m of an existing tidal feature (natural or man-made) may be treated with open spur ditches. This will permit operational treatment of isolated potholes near tidal features without having to extend closed or sill ditches close to these tidal sources, thereby minimizing the risk of non-tidal systems becoming directly connected to tidal sources (e.g. via muskrat burrowing).

Sill systems in salt hay zones may have their shallow outlets from 10-20 cm deep, depending on local tidal amplitude and soil composition. The DCMP-sponsored studies suggest that deeper sill depths (e.g. 20 cm) can be installed in areas of high tidal amplitude and peaty soil, whereas shallower depth sills (e.g. 10 cm) should be used in areas of low tidal amplitude and mineralogical soils.

An exception to avoiding creation of a water table elevation which averages a distance of 15 cm or more below local marsh surface could be made for low elevation areas that are subject to enough tidal surface flooding to retard colonization and/or growth of high elevation plants (e.g. in short *S. alterniflora* zones near tidal sources). In such areas, a 15 cm or greater water table displacement would be allowed, but only if created by open ditch drainage, not by spoil deposition on the marsh surface. Excessive deposition of spoil could raise surface elevations above heights where high marsh plants would no longer be suppressed by tidal flooding.

2) Efficient Dispersion and Use of Spoil

A second basic management goal is efficient use of spoil to fill

breeding depressions. Effort should be made in all OMWM systems designs to take advantage of spoil for beneficial filling of breeding depressions. Breeding areas filled with spoil will not require further modification. Precautions to take are not to fill depressions to heights above marsh surface and not to compact the fill too densely to prevent future plant growth.

3) Creating Natural-Looking OMWM Systems

A third basic management goal is creation of systems which look natural. Until the marsh surface is substantially revegetated following spoil deposition, a period of time usually taking one or two growing seasons, portions of the marsh will have an unavoidable muddy and/or barren look. After the vegetation has recovered, the positioning and configuration of the excavations will have the greatest impact on marsh aesthetics. The principal, long-term considerations for designing natural looking systems are to construct, whenever practical, irregular pond edges, islands in ponds, and curvilinear ditches. Geometric ponds (e.g. square or rectangular ponds) and long, straight ditches should be avoided.

INTERFACING OMWM WITH OTHER MARSH MANAGEMENT GOALS

The Delaware Division of Fish and Wildlife has embarked on a comprehensive marsh management program known as Integrated Marsh Management (IMM). . The purpose of IMM is to make sure that individual marsh management projects are not working at contradictory purposes and that projects with the potential to augment each other do so. Marsh management goals identified by the Division encompass environmentally-compatible mosquito control; waterfowl habitat enhancement, including selective creation or restoration of marsh ponds and optimum management of existing impoundments; *Phragmites* control; habitat conservation for fish spawning and nursery areas; habitat management for muskrat production and deer utilization; and integration of goals of the non-game and endangered species program (e.g. osprey production, protection of colonial waterbird nesting colonies and heronries, peregrine falcon hacking towers, etc.).

When the Mosquito Control Section performs OMWM, it has the potential to impact several of these other projects. Excessive spoil deposition or lowering of the subsurface water table could promote *Phragmites* growth, which must be avoided. OMWM activities detrimental to nesting sites of colonial waterbirds or raptors must be minimized. The creation of standing water on the marsh surface with OMWM (via sill and closed systems) can enhance habitat for waterfowl, wading birds, shorebirds, and muskrats. However, it must be understood that OMWM is first and foremost a mosquito control technique.

OMWM SYSTEM LONGEVITY

1) "Routine" Maintenance

Based on projections from New Jersey OMWM programs (Hansen *et al.* 1976), it is anticipated that "cleaning" (re-excavation) of most OMWM features will not have to be done more frequently than once every 15 to 20 years. **Open tidal ditches may require more frequent cleaning than sill or** closed systems, since sediment loads are deposited in the open ditches

twice per day. The shallow outlets of sill systems may also require more frequent maintenance (e.g. once every five years), but this cleaning could be rapidly and inexpensively accomplished because of the small areas and spoil volumes associated with sill outlets.

2) Corrective Actions and Preventive Measures

It may sometimes be necessary to return to a recently treated area in order to make corrections in either OMWM system design or installation. The two most likely problems to correct would be: 1) satisfactory mosquito reduction has not been achieved because of flaws in the site-specific OMWM design - additional, more intensive excavation is needed; 2) OMWM systems have been altered (e.g. surface ponds have drained) due to design flaws or animal damage - restoration of these systems must be done.

The most likely damage by animals is from snow goose grazing or muskrat burrowing. Snow goose creveys ("eat-outs") which produce mosquitoes should eventually be retreated with OMWM excavations. Muskrat burrowing damage can be lessened by terminating all sill or closed excavations no closer than 15 m from a tidal source in peaty soil and no closer than 8 m in mineral soil. To further prevent muskrat burrowing damage, or to repair drainage damage already done, barriers impervious to muskrat penetration (e.g. heavy-gauge fencing wire or plywood sheets) can be installed below the marsh surface between the end of a sill or closed system feature and an open tidal source (see Fig. 2). The barriers should extend one meter or deeper below marsh surface and extend laterally at least two meters to either side of a line between the end of the OMWM feature and the tidal source.

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INSECTS AND OTHER INVERTEBRATE PREDATORS

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INTRODUCTION

This chapter succeeds Collins and Washino (1985) and Legner (1985). It consists of 3 parts. Initially, the literature since the first edition is reviewed. The latest references cited there were published in 1980 and 1981, respectively. Legner (1995) refers to references until 1995 but the scope is different. Therefore, literature after 1981 is included here with a few earlier references when necessary. Invertebrate predators other than *Toxorhynchites* and copepods, which are covered in other chapters, are listed irrespective of their potential as biological control agents or pest status of prey mosquitoes. For the most part, only English-language references are included. Regarding biological control of rice field mosquitoes, Lacey and Lacey (1990) provide the complete literature including reports in Proceedings of California Mosquito Control Association, not cited here.

Second, predation by invertebrates and its significance in mosquito population dynamics are reviewed. This information is essential for ecologically sound manipulation of pest populations. Contribution of predation in egg and adult stages to dynamics of mosquito populations has not been evaluated quantitatively; therefore, only predation in the larval stage is included here. Larval predation is reviewed by 3 habitat types, namely, ground pools, natural containers (phytotelmata = small water bodies on living or dead plants) and artificial containers. Also included here are articles and reviews (Mogi 1981, Barr 1985, Service 1993) published after 1981, from which earlier publications can be traced.

Third, difficulties and possibilities of using invertebrate predators for mosquito control are discussed.

ADULT PREDATION

Despite accumulation of data about adult survival rates in the field (Service 1993), mortality factors have rarely been quantified. Daily mortality of newly emerged *Culex tritaeniorhynchus* adults by hunting spiders (*Pirata piraticus* most abundant) in rice fields was estimated to be 20% (Takagi et al. 1996) but this may be an overestimate due to the impact of artificial enclosure. Mosquitoes comprised 32% and 25% of prey of *Hygropoda dolomedes* and *Dendrolycosa* sp. (Pisauridae), respectively, both being spiders that make sheet webs at the top of leaves (Cerveira and Jackson 2002). Females of *Crossopriza lyoni* (Pholcidae), a web-making spider common in and around houses, ate, on average, one *Aedes aegypti* or *Anopheles dirus* per day in the laboratory (Strickman et al. 1997). Adults of *Ae. aegypti* adhered to a web of *Theridion rufipes* (Theridiidae) and were immediately paralyzed by toxic substances on the thread (Fox 1998). A jumping spider *Salticus scenisus* (Salticidae) caught blood-fed females of *Anopheles gambiae* effectively in the laboratory because of slow flight speeds (Roitberg et al. 2003). Among natural prey of a house-dwelling jumping spider *Evarcha culicivora* (Salticidae), 70% were female mosquitoes including many anophelines (probably *An. gambiae*) (Wesolowska and Jackson 2003).

In container-type larval habitats, adult mosquitoes are caught by predators above the water. Larvae of *Cx. restuans* and *Anopheles* spp. in tires with webs of spiders (predominantly *Agelenopsis naevia* of Agelenidae) were only 8% as abundant as those in tires without webs (Ramoska and Sweet 1981). A hunting spider *Heteropoda venatoria* (Sparassidae) preyed on *Ae. albopictus* in artificial containers (Sulaiman and Jeffery 1986). A jumping spider *Paracyrba wanlessi* (Salticidae) in bamboo internodes caught newly emerging adult mosquitoes (Zabka and Kovac 1996). In bamboo internodes, adult mosquitoes were prey of a predacious bug *Emesopsi streiti* (Reduviidae) (Kovac and Yang 1996) and web-making spiders belonging to several families (several species of Theridiidae were dominant) (Kovac and Streit 1996). Vellids inhabit tropical phytotelmata such as tree holes and bromeliad axils (Polhemus and Polhemus 1991, Polhemus and Copeland 1996, Fincke 1999) and bamboo internodes (Yang and Kovac 1995). *Microvelia cavicola* and *Paravelia myersi* (Vellidae) fed on adult mosquitoes emerging in tree holes (Yanoviak 2001b). The larva of a mycetophilid fly *Xenoplatyura beaveri* eats emerging adult mosquitoes caught by a sticky web made in *Nepenthes* pitcher plants (Mogi and Chan 1996).

Dragonflies *Pantara hymenaea* and *Erythemis collocate* attack swarming of *An. freeborni* after sunset (Yuval and Bouskila 1993).

EGG PREDATION

The imported fire ant *Solenopsis invicta* ate or damaged terrestrial eggs of *Ae. albopictus* and *Psorophora columbiae* in laboratory and field exposure experiments (Lee et al. 1994, Burnham et al. 1994). Eggs of *Ae. aegypti* in subterranean habitats were consumed by the American cockroach *Periplaneta americana* but not by ants (Russell et al. 2001).

LARVAL AND PUPAL PREDATION

Predacious Mosquitoes other than *Toxorhynchites*

Species of the *Aedes* subgenus *Alanstonea* (Ramalingam and Ramakrishnan 1971, Mogi and Chan 1996), the genus *Lutzia* (Hong 1982, Thangam and Kathiresan 1996), the *Ochlerotatus* subgenus *Mucidus* (Mattingly 1961), and the *Psorophora* subgenus *Psorophora* (Carpenter and LaCasse 1955) are obligatory predators. Generally they inhabit ground pools except for 2 *Alanstonea* species specialized to *Nepenthes* pitcher plants. In temporary pools, 47% of 4th instar *Ps. ciliata* larvae had *Oc. albifasciatus* remains in the guts (Campos et al. 2004).

Obligatory or facultative predacious habits are reported for some species of *Anopheles* (Copeland and Craig 1992, Nannini and Juliano 1998), *Culex* (Yanoviak 2001a), *Eretmapodites* (Lounibos 1980), *Topomyia* (Ramalingam 1983, Okazawa et al. 1986, Miyagi and Toma 1989), the *Tripteroides* subgenus *Rachisoura* (Lee et al. 1989) and New World Sabethini genera *Johnbelkiana*, *Sabethes*, *Trichoprosopon* and *Wyeomyia* (Machado-Allison et al. 1985). The list could be enlarged with more observation in the tropics where mosquito diversity is high. All of these species inhabit phytotelmata.

Predacious mosquito larvae prey on other mosquitoes and smaller conspecifics. Their roles in control of other mosquitoes have seldom been evaluated.

Diptera other than Mosquitoes

Chaoborid larvae are obligatory predators for aquatic macro-invertebrates including mosquito larvae. Generally they inhabit ground pools and large containers (McLaughlin 1990, Morrison and Andreadis 1992, Sunahara et al. 2002). Predation by *Mochlonyx cinctipes* was a primary factor for 1st instar mortality of several *Ochlerotatus* species in snowmelt pools (Morrison and Andreadis 1992). Third and 4th instars of *Chaoborus americana* ate 1st and 2nd instars of *Aedes vexans* (Helgen 1989). Some species of *Corethrella* inhabit phytotelmata such as tree holes (Lounibos 1983), bromeliad axils (Frank and Curtis 1981), pandanus and palm axils (Belkin 1962), and *Nepenthes* pitcher plants (Beaver 1983, Clarke and Kitching 1993), together with mosquito larvae. Predation by *Corethrella appendiculata* was an important mortality factor for *Aedes triseriatus* in tree holes (Lounibos 1983, Bradshaw and Holzapfel 1985).

Pentaneura sp. (Chironomidae) in *Heliconia* flower bracts keeps *Wyeomyia pseudopecten* densities low and prevents colonization of highly vulnerable *Trichoprosopon digitatum* (Naeem 1988). *Metriocnemus knabi*, cohabiting with *Wy. smithii* in *Sarracenia* pitcher plants, had long been regarded as a detritivore but proved to be a predator of non-*Wyeomyia* mosquito larvae (Petersen et al. 2000). *Anatopynia pennipes* of tree holes ate mosquito larvae in the laboratory (Kitching 1983). Other nematoceran predators of mosquito larvae in phytotelmata include species of *Bezzia* and *Culicoides* (Ceratopogonidae) (Hribar and Mullen 1991, Yanoviak 2001b), and *Sigmatomera* (Tipulidae) (Fish 1983, Yanoviak 2001b).

Among brachyceran larvae in phytotelmata, predation on mosquito larvae is reported for *Stenomicra* (Periscelididae) (Fish 1983) and 2 *Ocyptamus* species (Syrphidae) (Rotheray et al. 2000) in bromeliad axils; *Graphomyia* species (Muscidae) in *Curcuma* flower bracts (Kitching 1990) and bamboo stumps (Kovac et al. 1997); and species of *Phaonia* (Muscidae), *Nepenthomyia* (Calliphoridae), *Pierretia* (Sarcophagidae) and *Nepenthosyrphus* (Syrphidae) in *Nepenthes* pitcher plants (Mogi and Chan 1996).

Obligatory or facultative predator species would be found more commonly among dipteran larvae with more extensive observation.

Coleoptera

Adults and larvae of Dytiscidae and larvae of Hydrophilidae are common predators in ground pools. Predation by dytiscid and hydrophilid larvae in artificially flooded ponds (Walton et al. 1990), dytiscid larvae in ephemeral pools (Reisen et al.1989) and hydrophilid larvae in rice fields (Bence 1982) was an important mortality factor for *Cx. tarsalis*. In snowmelt pools of boreal regions or highlands, dytiscids may be the most abundant predators and make assemblages comprised of several guilds, each including multiple species (Larson 1990, Nilsson and Svensson 1994). They can reduce mosquito densities in some pools (Stout 1982, Nilsson and Söderström 1988, Nilsson and Svensson 1994, Lundkvist et al. 2003) but their role in mosquito control may be limited by incomplete habitat overlap, preference for alternative prey such as *Daphnia*, and self-regulation of densities by cannibalism and emigration (Stout 1982, Juliano and Lawton 1990, Lundkvist et al. 2003).

Dytiscid and hydrophilid beetles were reported from phytotelmata such as tree holes, leaf axils of bromeliads, bananas and taros, flower bracts of *Heliconia*, bamboo stumps, fallen palm bracts and fallen leaves (Naeem 1990, Kitching 2000, Greeney 2001, Yanoviak 2001b, Mogi 2004). They are regarded as facultative or accidental inhabitants. A rove beetle *Acylophorus* sp. specialized to bamboo internodes preys on larval and pupal mosquitoes at the water surface (Kovac and Streit 1996).

Notonectidae

Both nymphs and adults of Notonectidae are well known as efficient predators for mosquito larvae in ground pools. Ecological studies have concentrated on species of the genus *Notonecta* in temperate regions, and studies for other genera (Wattal et al. 1996, Eitam et al. 2002) and in the tropics (Wattal et al. 1996) are fewer.

Notonectids generally prefer mosquitoes to chironomids, ceratopogonids, cladocerans, etc. (Murdoch et al. 1984, Blaustein 1998, Eitam et al. 2002, Blaustein et al. 2004), but alternative prey may be preferred (Chesson 1989) and constitute the primary components of natural diets (Giller 1986). Selectivity is influenced by prey-predator size combination (Scott and Murdoch 1983, Wattal et al. 1996), mosquito species (Sih 1986, Blaustein et al. 1995, Wattal et al. 1996) and predator niches differing among species (Giller and McNeill 1981). Habitat structure also influences predation efficiency. Floating and emergent macrophytes are generally regarded as refuges from aquatic predators (Orr and Resh 1989), but, in some situations, algal mats did not provide *An. quadrimaculatus* protection from notonectids (Wallace and Merritt 1999). Evaluation of effectiveness of mosquito control in nature is complicated further by the fact that some mosquitoes vulnerable to *Notonecta* and *Anisops* avoid laying eggs in waters infested with these predators (Chesson 1984, Eitam et al. 2002, Kiflawi et al. 2003, Eitam and Blaustein 2004, Blaustein et al. 2004). Simulation models suggest that *Culiseta longiareolata*, though susceptible to *Notonecta maculata* predation, can be abundant owing to strong avoidance for water containing the predator (Spencer et al. 2002).

Hemiptera other than Notonectidae

Species of Belostomatidae (Venkatesan and Jeyachandra 1985), Naucoridae (McCoull et al. 1998), Nepidae (Ambrose et al. 1993), Vellidae (Miura and Takahashi 1988a) and Gerridae (Ambrose et al. 1993, Spence and Andersen 1994) prey on mosquito larvae in the laboratory. The latter 2 are semi-aquatic and prey on mosquito larvae at the water surface. In addition to those families, aquatic or semi-aquatic hemipteran predators inhabiting rice fields include Saludidae, Hebridae, Mesoveliidae, Hydrometridae, Ochteridae and Pleidae (Yano et al. 1981). Hemipteran predators mainly inhabit ground pools but some vellid species occur in phytotelmata as stated in the adult predation section.

Confirmed or potential predators for mosquitoes are diverse in Hemiptera but their roles in controlling mosquitoes have rarely been evaluated in nature. Large domestic containers with *Micronecta quadristrigata* (Corixidae) were positive for *Ae. aegypti* and *Ae. albopictus* less frequently (Nam et al. 2000), but capacity of corixid bugs as mosquito predators has not been well documented.

Odonata

Predation ability of local species of dragonflies (Urabe et al. 1986a, Ree and Lee 1982, Thangam and Kathiresan 1996) and damselflies (Miura and Takahashi 1986b) inhabiting ground pools was evaluated in the laboratory.

Naiads of a univoltine dragonfly *Sympetrum frequens* hatch from eggs in rice fields flooded in May and are abundant until late June when adults emerge (Urabe et al. 1986b, 1990). In some rice fields, daily mortalities of *An. sinensis* larvae due to predation by naiads were estimated to be >90% in June when other predators were sparse (Urabe et al. 1986b). Average mortalities for larger areas were lower due to variation in naiad densities but were still as high as 40% (Urabe et al. 1990). Because naiad densities in respective rice fields are determined primarily by densities of dormant eggs laid in shallow water during the preceding autumn, a possibility of manipulating naiad densities by post-harvest water management was suggested (Urabe et al. 1990).

Predation by damselfly naiads was a main mortality factor for *Cx. tarsalis* in a stable wet pasture (Reisen et al. 1989). Foreguts of several species of dragonflies and damselflies inhabiting roots of water lettuces *Pistia striates* contained remains of *Mansonia* larvae (Lounibos et al. 1990).

Naiads of dragonflies and damselflies are not rare in phytotelmata such as tree holes, leaf axils, bamboo stumps and internodes in the tropics (Corbet 1983, Orr 1994, Louton et al. 1996, Copeland et al. 1996, Fincke et al. 1997, Fincke 1999, Kitching 2000). They reduce mosquito densities in tree holes (Copeland et al. 1996, Fincke et al. 1997).

Naiads of a dragonfly *Crocothemis servilia* were mass produced with mosquito larvae as prey and released regularly into artificial containers in a village of Yangon with resultant reduction of both larval and adult indices of *Ae. aegypti* (Sebastian et al. 1990).

Culiseta longiareolata avoids oviposition in experimental pools with naiads of a large dragonfly *Anax imperator* (Stav et al. 1999, 2000). Such repulsion, if common with other combinations of

Odonata and mosquito species, would complicate evaluation of the total impact of Odonata on mosquito abundance.

Araneae

Hunting spiders *Dolomedes triton* (Pisauridae), and *Pirata sedentaris* and *Pardosa delicatula* of Lycosidae readily preyed on *Cx. pipiens* released in large containers simulating ponds (Breene et al. 1988). Efficiency of aquatic and semi-aquatic spiders as predators for larvae of *An. messeae* and *Cx. modestus* was evaluated in the laboratory (Perevozkin et al. 2004). Among 3 genera hunting at the water surface, *Dolomedes* was a more efficient predator than species of *Pirata* and *Pardosa*. *Argyroneta aquatica* (Argyronetidae), making webs under water, effectively caught mosquito immatures, especially those of *An. messeae*. Mosquito immatures are regarded as main prey for *Dolomedes* and *Argyroneta*.

A jumping spider *Paracyrba wanlessi* (Salticidae) in bamboo internodes catches mosquito immatures at the water surface (Zabka and Kovac 1996).

Acari

Free-living nymphs and adults of water mites (Hydrachnida) are predacious or omnivorous. Adult females of *Encentridophorus similes* (Unionicolidae) preferred 1st instar *Ae. albopictus* in the laboratory, although this mite inhabits ground pools in nature (Rajendran and Prasad 1989). Nymphs and adults of a water mite *Arrenurus madaraszi* (Arrhenuridae) ate 1st instars of *Ae. albopictus*, *Ae. vittatus* and *Ar. subalbatus* in the laboratory (Rajendran and Prasad 1994). A colony of this mite was maintained with adults of those mosquitoes as hosts for the larvae, though only *Anopheles* inhabiting ground pools are natural hosts.

Water mites mainly inhabit ground pools but some were recorded from phytotelmata. Species of Arrhenuridae were found from tree holes and bromeliad axils (Kitching 2000). Larvae of Hydryphantidae were parasitic on adult mosquitoes emerging from tree holes, indicating the occurrence of their nymphs and adults there (Williams and Proctor 2002).

Crustacea other than Cyclopoida

The Californian tadpole shrimp *Triops newberryi* (=*T. longicaudatus*) (Triopsidae, Nostraca) was an effective predator of *Cx. quinquefasciatus* and *Cx. tarsalis* larvae in laboratory and field experiments (Tieze and Mulla 1989, 1990). *Culex quinquefasciatus* laid fewer eggs in containers with tadpole shrimps in the laboratory, and egg rafts, as well as larvae and pupae, of *Cx. tarsalis* were fewer in ponds naturally inhabited by tadpole shrimps (Tieze and Mulla 1991). For mass production and effective introduction of tadpole shrimps into field sites, influences of several environmental factors on egg hatch, larval development, longevity and egg production were examined (Fry and Mulla 1992, Fry-O'Brien and Mulla 1996, Su and Mulla 2001, 2002a). Egg densities in flood-irrigated date gardens were high at ranches on clay loam soil and not disked recently (Su and Mulla 2002b).

Tadpole shrimps introduced into artificial ponds as eggs or adults colonized successfully and persisted through repeated flooding for 1 year with reduction of *Cx. tarsalis* densities (Fry et al. 1994). Tadpole shrimps, hatching immediately in flooded ponds, could reduce abundance of early colonizing *Cx. tarsalis* prior to colonization of insect predators (Walton 2001). Even the more quickly developing *Ps. columbiae* could be reduced effectively in a date garden where tadpole shrimp eggs or adults were introduced 1 year before and had been subjected to ordinary flood irrigation and disking (Su and Mulla 2002c). On the one hand, tadpole shrimps may shorten mosquito larval durations because they stir up the mud and increase suspended particles available to mosquito larvae (Tieze and Mulla 1990).

A freshwater prawn *Macrobrachium borellii* proved to be a potential predator for *Cx. pipiens* s.l. (Collins 1998). The Louisiana red swamp crawfish *Procambarus clarkia*, introduced in Africa and common in Kenya, proved to be a potential predator for *An. gambiae* (Mkoji et al. 1999). Guts of this crawfish in Kenya contained remnants of mosquito larvae (Smart et al. 2002).

Annelida

Some species of leeches (Hirudinea) are predacious. A leech *Helobdella triserialis lineate* preyed on *Ae. fluviatilis* and *Cx. quinquefasciatus* larvae and deterred ovipisition of these mosquitoes in the laboratory (Consoli et al. 1984, cited from Tieze and Mulla 1991).

Flatworm

Since the late 1970s, a few species of turbellarian flatworms have been studied extensively in the USA as agents for biological control of mosquitoes (Legner 1985, 1995). Furthermore, species of *Dugesia* (Tricladida), as predators for mosquitoes, were evaluated in the laboratory in Canada (George et al. 1983), Brazil (Melo and Andrade 2001) and Malaysia (Loh et al. 1992). *Dugesia tigrina* kills mosquito larvae by direct capture or by trapping in secreted mucus (Meyer and Learned 1981, Mckee et al. 1997).

In field experiments, *Dugesia dorotocephala* in ponds (Ali and Mulla 1983) and *Dugesia tigrina* in catch basins (George et al. 1983) effectively reduced *Culex* larvae. These planarians tolerate some formulations of organophosphate and pyrethroid insecticides (Nelson et al. 1988), *Bacillus thurengensis israelensis* (*Bti*) (Perich et al. 1990) and insect growth regulators (IGRs)

(Nelson 1981, Nelson et al. 1986, Nelson et al. 1988, Nelson et al. 1994) at dosages lethal to mosquito larvae. For mass production, manual mechanical sectioning using glass cover slips was more effective than production of cocoons containing eggs (Callahan and Morris 1989). Large individuals could be stored at 10°C for 231 days with low mortality (Callahan and Morris 1989). Possibility of dispensing planarians (>15 mm) by sprayers was examined by Darby et al. (1988).

In laboratory experiments *Dugesia dorotocephala* did not affect survival of 3 species of Crustacea, 2 species of Mollusca, a leach (Hirudinea), dragonfly and damselfly (Odonata), caddis fly (Trichoptera), water strider (Gerridae), tadpole, *Gambusia affinis*, or fry of bluegills and fathead minnows, but caused 100% mortality within 1 week of a roundworm (Oligocaeta), *Cyclops* sp. and *Tx. amboinensis* that rarely encounter flatworms in nature (Perich and Boobar 1990).

Species of *Mesostoma* (Rhabdocoela) kill larvae of *Aedes*, *Anopheles*, *Culex* and *Culiseta* in the laboratory (Kolasa 1984, Blaustein 1990, Blaustein and Dumont 1990, Wrona and Koopowitz 1998). Besides direct capture and trapping in mucus, killing by toxins released into water was observed for *Mesostoma* cf. *lingua* (Dumont and Carels 1987) but not confirmed for *Mesostoma ehrenbergii* (Wrona and Koopowitz 1998). In some rice fields, predation by *Mesostoma* was regarded to be a main determinant of *Cx. tarsalis* and *An. freeborni* densities (Blaustein 1990, Blaustein and Dumont 1990). Three species of *Mesostoma* from Australia, Africa and Papua, New Guinea reproduced faster than *Dugesia* in the laboratory (Kolasa 1987).

LARVAL HABITAT AND MORTALITY FACTOR

Ground Pools

Ground pools include all kinds of larval mosquito habitats on the ground, ranging from transient rainwater pools at the one extreme to permanent ponds at the other, either natural or artificial. Some artificial ponds are constructed in place of natural habitats. Some types of habitats, for example rice fields, include a variety of pools covering a wide range on the temporary-permanent continuum. Some running water habitats are also included because mosquito larvae occur in stagnant pools along margins.

Generally, mortalities of larval mosquitoes are high, >90% being common as reported for vegetated dam margins (Rae 1990), large artificial shallow ponds (McDonald and Buchanan 1981), man-made and natural marshes (Orr and Resh 1989), semi-permanent pools, flooded grasslands and temporary pools (Mottram and Kettle 1997), swamps and pools produced by irrigation (Aniedu et al. 1993), swamps, rock pools and puddles (Edillo et al. 2004), rice fields (Andis and Meek 1985, Mogi et al. 1984, 1986; Urabe et al. 1986b, 1990), temporary ponds (Renshaw et al. 1993), creeks (Mogi and Okazawa 1990), ponds, rice fields, streams and rivers (Gunasekaran 1994), water courses and seepage pools (Reisen et al. 1982), a stable wet pasture and man-made ephemeral pools (Reisen et al. 1989), open transient floodwater pools (Campos and Sy 2003), temporary pools (Casanova and do Prado 2002), snowmelt temporary pools (Morrison and Andreadis 1992), and drains, cesspits, wells, cesspools and cisterns (Menon and Rajagopalan 1981). Many species studied in these reports were *Culex* with some *Anopheles* and *Aedes*.

Abiotic mortality factors may be important in some habitats under some situations (Menon and Rajagopalan 1981, Reisen et al. 1989, Mogi and Okazawa 1990, Renshaw et al. 1993, Casanova and do Prado 2002). Among biotic factors, overcrowding was significant in some cases (Menon and Rajagopalan 1981, Renshaw et al. 1993). For *Oc. albifasciatus* in temporary pools, a Nematoda parasite was considered important (Campos and Sy 2003) as well as insect predators (Campos et al. 2004). In more cases, however, predation was regarded the most important cause of death.

In relatively lasting habitats, fish predation may be most important (Yu et al. 1981, Lounibos et al. 1992). It is consistent with a generalized schema illustrating the shift in the top predator from invertebrates to fish on the temporary-permanent gradient in freshwater habitats (Wellborn et al. 1996). Presence of fish, however, does not always mean desirable impacts on mosquito abundance (Kramer et al. 1987, Blaustein 1992, Nielsen et al. 1999, Greenway et al. 2003). Invertebrate predators, as a whole, are more ubiquitous than fish in relation to habitat types because of their ecological diversity, small size, short generation time, and, for insects, ability of aerial colonization. Examples documenting the importance of specific predator groups were mentioned in respective sections above. In addition, occurrence of predacious copepods was negatively associated with densities of *An. albimanus* in ponds (Marten et al. 1989) and *An. quadrimaculatus* in rice fields (Marten et al. 2000). In many cases, however, predator complexes that include many groups cause high mortalities (McDonald and Buchanan 1981, Menon and Rajagopalan 1981, Mogi et al. 1984, 1986; Rae 1990, Aniedu et al. 1993, Mottram and Kettle 1997, Casanova and do Prado 2002).

Small temporary pools have customarily been regarded as rather predator-free. Fish can neither enter small isolated pools nor survive there. Small

pool sizes may limit aerial colonization of some insect predators (Stout 1982, Larson 1990, Pearman 1995, Nilsson and Svensson 1995, Sunahara et al. 2002, Lester and Pike 2003). In experimental temporary pools without predators, mosquitoes were suppressed by competitors (Chase and Knight 2003), but generalization may not be warranted. Although more species, including insect predators, are generally present in larger and more lasting pools (Ranta 1982, Roth and Jackson 1987, Collinson et al. 1995, Schneider and Frost 1996, Schneider 1997, Spencer et al. 1999, Baber et al. 2004), there is increasing evidence for the presence of predators even in small and ephemeral habitats (Rosenberg 1982, Williams 1997, Brendonck et al. 2002, Eitam et al. 2004). Rain pools in an urban park were habitats of insect predators as well as mosquitoes (Fischer et al. 2000). Rain pools on newly deforested lands were colonized immediately by insect predators together with mosquitoes (Mogi et al. 1999). Functional and taxonomic components of invertebrate communities in temporary pools have considerable similarity throughout the wide geographical range, with Coleoptera and Hemiptera as the most diverse predator groups (Williams 1997).

Although preference for some prey types is common, invertebrate predators in ground pools are polyphagous. Many of them are also rather general in their habitat selection.

Natural Containers

This category indicates phytotelmata, water collections on living or dead plants (Frank and Lounibos 1983, Kitching 2000, Greeney 2001).

Tree holes are common from temperate through tropical regions, and thus have been studied most. Nevertheless, quantitative evaluation of mosquito mortalities is almost limited to the USA. Predation by *Tx. rutilus* and *Co. appendiculata* keeps *Ae. triseriatus* densities low (Lounibos 1983, 1985; Lounibos et al. 1997, Bradshaw and Holzapfel 1985), while predation-resistant *Orthopodomyia signifera* occurred more frequently in holes with predators (Lounibos 1983, Bradshaw and Holzapfel 1985). Pupation success varied from 0 to 60% in tree holes for *Ae. sierrensis* in the presence of several species of micro-parasites including *Lambornella clarki* (Hawley 1985a). *Aedes africanus* suffered 71–100% immature mortalities with predation by *Tx. brevipalpis* being a main cause of mortality (Sempala 1981). Tree holes in the tropics may be inhabited by a variety of predators (Kitching and Orr 1996, Fincke et al.1997, Fincke 1999, Yanoviak 2001b). Tree holes in Panama, for example, are inhabited by Odonata naiads, including several species of dragonflies and damselflies, *Tx. theobaldi*, *Tr. digitatus*, tadpoles of *Dendrobates auratus* as aquatic predators and vellids as semiaquatic predators (Fincke et al. 1997).

Phytotelmata other than tree holes may also be habitats of multiple predator species as observed for bamboo stumps and internodes (Okazawa et al. 1994, Kovac and Streit 1996, Louton et al. 1996), fallen nut capsules (Caldwell 1993), and *Nepenthes* pitcher plants (Mogi and Yong 1992, Clarke and Kitching 1993, Mogi and Chan 1997). Evaluation of mosquito mortality in these phytotelmata is limited despite their diversity. Immature mortalities were 47–88% for *Ae. simpsoni* in *Colocasia* leaf axils but higher than 82% in axils of *Xanthosoma* and bananas, and overcrowding was suggested as a cause of mortality (Lutwama and Mukwaya 1995). Addition of *Tx. haemorrhoidalis* reduced mosquito densities and mean larval ages in *Heliconia* flower bract and bamboo stumps (Lounibos et al. 1987). In *Alocasia* leaf axils, *Tx. splendens* reduced pupa/larva ratios of mosquitoes and it was estimated that predation halved adult production even if predator-free axils were included (Mogi et al. 1985). Even in very small habitats, specialized predators may reduce mosquito density, as exemplified by *Pentaneura* sp. in *Heliconia* flower bracts (Naeem 1988).

Invertebrate predators in natural containers are generally polyphagous but are usually specialists in particular habitat types. Numbers of predator species are distinctly fewer than in ground pools. In ground pools, >20 predator genera, many of them including multiple species, may be present (Williams 1997). As for phytotelmata, the maximum may be ca. 20 species (excluding spiders above the water) recorded in the 4-year survey of 206 natural and 153 artificial tree holes of Barro Colorado Island, Panama (Yanoviak 2001b). In the absence of fish, invertebrates are the only predators except in some tropical phytotelmata with predacious tadpoles. For one habitat type in one locality, predator species are usually less than 5 and occasionally none. Predator occurrence in each habitat unit (e. g., one tree hole) is further limited by habitat patchiness. When 4 predator species occur in tree holes locally, frequencies with 4, 3, 2 and 1 predator species were 1, 5, 7 and 11 for 24 observations (6 holes × 4 times) (Kitching 1987). Units with fewer predators result from both biological (species interaction, microhabitat preference) and stochastic processes as aggregation with an intensifier (Shorrocks 1990).

Predation in natural containers differs from that in ground pools in 2 aspects, both arising from the small size of natural containers. First, it is common that a single individual alone can survive in a single habitat unit due to intra- and inter-specific predation. In fallen nut capsules with 111 ml water on average, for example,

a single predator, either *Dendrobates* tadpole, *Microstigma* naiad or *Toxorhynchites* larva, survives (Caldwell 1993). A primary determinant of outcomes of predator encounters is body size (e. g., Lounibos 1985, Caldwell 1993) but *Tx. theobaldi* was often killed by smaller odonates and tadpoles (Fincke 1999).

Secondly, predation in natural containers may favor mosquitoes by mitigating competition for food. Experiments with microcosms simulating tree holes demonstrated that food available for *Ae. triseriatus* is limited (e. g., Fish and Carpenter 1982, Carpenter 1983, Leonard and Juliano 1995, Walker et al. 1997) and that density reduction by *Tx. rutilus* results in faster development, larger size and higher female ratios (Chambers 1985, Grill and Juliano 1996). In tree holes with *Co. appendiculata*, pupal proportions of *Ae. triseriatus* were higher due probably to diminished competition (Lounibos 1985). Pupal weight and adult life expectancy of *Ae. sierrensis* inhabiting tree holes were reduced at high densities, suggesting the highest vectorial capacity at intermediate larval densities (Hawley 1985b). Size reduction due to overcrowding was observed also in taro leaf axils (Mogi 1984, Mogi et al. 1985).

Artificial Containers

This category indicates all kinds of man-made containers, either for water storage or discarded, in and around human houses.

Despite the importance of man-made containers for production of vector mosquitoes, quantitative evaluation of immature mortalities is limited. Indoor water storage pots produced more *Ae. aegypti* pupae after contamination by children because of food replenishment (Subra 1981). Mortality of *Ae. aegypti* immatures ranged from 5–79% by container types and locations but was usually higher than in the laboratory, with overflowing being a cause of larval loss in outdoor containers (Tun-Lin et al. 2000). In rain barrels, *Cs. incidens* suffered 97% larval mortality due probably to food shortage (Barr 1985). In all these cases, there were no predators. In used milk tins in rubber estates, larval mortalities of *Ae. albopictus* were 74–80% (Sulaiman and Jeffery 1986). In this case, a few predators were present, but competition for resources was considered most important among factors limiting mosquito production.

Tree holes and other phytotelmata on plants cultivated in residential areas are often predator-free. These sites may provide mosquitoes primarily using artificial containers with concealed secondary habitats (Mogi 2000). Some recent examples are *Ae. aegypti* in tree holes, bamboo and papaya stumps, leaf axils and fruit shells in the Caribbean (Chadee et al. 1998), *Ae. albopictus* in bromeliad axils in Florida (O'Meara et al. 1995, 2003) and *Ae. aegypti* (Maguire et al. 1999) and *Ae. notoscriptus* (Foley et al. 2004) in bromeliad axils in northern Queensland.

Most *Ae. aegypti* adults emerging from containers are smaller than adults reared with ample food, indicating limited resources (Tun-Lin et al. 2000, Strickman and Kittayapong 2003). Large adults emerged from containers with fewer larvae or more resource input (Strickman and Kittayapong 2003). Larger females are generally believed to have higher potential of disease transmission (e. g., Nasci 1986, Sumanochitrapon et al. 1998) despite some doubts (Scott et al. 2000).

DIFFICULTY OF USING INVERTEBRATE PREDATORS

Many invertebrates have been noticed or investigated because of their potential as biological control agents. Among those discussed in this chapter, dragonfly naiads were used for a village scale trial, but until now none have been incorporated into control programs as essential components.

One obstacle for practical use is, as pointed out repeatedly, a difficulty in mass production, storage and release of those predators; they are usually cannibalistic and require living organisms as food.

The second and more fundamental difficulty arises from polyphagy of invertebrate predators. Polyphagy has both advantages and disadvantages. An advantage is that such predators can persist when mosquitoes are temporarily sparse or absent (Murdoch et al. 1985). A disadvantage is that they may not reduce mosquitoes because of availability of alternative prey (Bence 1988). Removal of competitors by predators could even favor mosquitoes; *Cx. tarsalis* larvae developed better in cages in rice fields with *Gambusia affinis* because removal of Cladocera increased food for mosquitoes (Blaustein and Karban 1990).

The third and also fundamental difficulty arises from presence of indigenous predators, both invertebrates and vertebrates. Fish usually reduce the abundance of insect predators (Miura et al. 1984, Morin 1984a, El Safi et al. 1985, Bence 1988, Kramer et al. 1988, Blaustein 1992). Odonate naiads reduce dytiscid densities in larger bog pools (Larson 1990). Vulnerability to other predators differs among species. Dragonflies and damselflies have evolved anti-predator behaviors against the top predator in their habitat, either fish or large dragonfly (Johnson 1991). Anti-predator behaviors may differ among species within a genus such as *Enallagma* damselflies (McPeek 1990a, 1990b; McPeek et al. 1996) and *Notonecta* backswimmers (Cook and Streams 1984) and even within a single species such as the dragonfly *Pachydiplax longipennis* (Hopper

2001). Size-dependent selective fish predation on dragonflies may favor small and/or late colonizing species (Morin 1984b). Interaction among predators could be more severe in small phytotelmata as stated already.

Besides direct predation, predators may interfere through chemical or other cues. A hydrophilid *Tropisternus lateralis* (Resetarits 2001) and a phantom midge *Chaoborus albatus* (Petranka and Fakhoury 1991) avoid laying eggs in pools with fish. Contrastingly, in experimental pools exposed to aerial colonization, insect predators were reduced when fish were free-roaming but not when fish were caged, indicating the importance of direct predation (Åbjörnsson et al. 2002). Interference occurs also in aquatic stages. Movement of dragonfly naiads was suppressed by waterborne cues of fish (Hopper 2001). Feeding activity of damselfly naiads was reduced by chemical cues from fish or hemipteran predators (Koperski 1997). Predacious *Notonecta glauca* suppressed activity of damselfly naiads but nonpredacious *Corixa punctata* did not despite similar size, shape and behavior (Heads 1985). Activity of naiads of a dragonfly *Erythemis simplicicollis* was suppressed by the presence of the other dragonfly *Tramea lacerate*, so the impact on the common prey was not additive (Wissinger and McGrady 1993). Chemical and other type interference among predators in nature is yet to be evaluated.

Predators may facilitate effectiveness of other predators. A hydrophilid *Tropisternus lateralis* reduced *Cx. tarsalis* survival more when *Gambusia affinis* was present, probably because fish preferably removed smaller invertebrate prey and forced the hydrophilids to concentrate on mosquitoes (Bence 1982).

In natural communities, many species of predators and prey interact with each other in complicated manners (e. g., McPeek 1996 for interactions among fish, dragonflies and damselflies). Therefore, impacts of introduced polyphagous predators on target mosquitoes are neither expected to be stable nor predictable with certainty. Potential of candidate predators may be evaluated first in the laboratory with and without alternative prey, then in the field by using enclosures with and without those predators. However, this type of evaluation is useful only when repeated introductions directly into each pool or container are planned. The total effect at the population level results from distribution and abundance of candidate species and of target mosquitoes in heterogeneous environments. Escape from predators due to incomplete overlap can result from differences in seasonality, reproductive capacity, microhabitat preference, etc. In patchy habitats such as scattered small ground pools, phytotelmata and artificial containers, stochastic processes intensified by aggregation can yield predator-free habitat units (Shorrocks 1990). Avoidance by mosquitoes of water containing invertebrate predators is common, as reported for backswimmers, dragonflies, tadpole shrimps and leeches (see respective sections). It makes prediction and evaluation of predator impact further complicated (Chesson 1984).

Notonecta and *Gambusia affinis* were listed as examples of successful biological control of mosquito larvae with polyphagous predators (Murdoch et al. 1985), but only the latter has been actually used. There has been increasing controversy about its effectiveness and environmental impacts.

POSSIBILITY OF USING INVERTEBRATE PREDATORS

The preceding discussion leads to 2 strategies of using invertebrate predators for mosquito control. One is to fill vacant niches. The other is to maximize effectiveness of local predators.

To fill vacant niches, agents have to be newly introduced. By accidental introduction, 3 damselfly and 4 dragonfly species colonized in the Hawaiian Islands without noticeable adverse impacts on native *Megalagrion* damselflies, at least in Oahu (Englund 1999). In view of growing public refractoriness to introduction of exotic species, however, the best candidates are local species absent from or less abundant in target mosquito habitats.

Vacant niches occur in artificial containers usually lacking predators. It is reasonable that village scale trials or operations using invertebrate predators (*Toxorhynchites*, predacious copepods and dragonfly naiads) have targeted mosquitoes in artificial containers in residential areas. As water is stored in containers for domestic use, predators may be preferred to chemicals or microbes. Regardless of control measures, however, larval density reduction in artificial containers with limited food input may not proportionally reduce disease transmission risks due to mitigated competition and, in the worst case, may increase risks by production of larger and stronger adults (Agudelo-Silva and Spielman 1984).

Vacant niches may also occur in newly constructed pools. Tadpole shrimp *Triops newberryi* hatching from dormant eggs immediately after flooding can control early colonizing *Cx. tarsalis* more effectively than late colonizing insect predators (Walton 2001). This predator can persist for a few generations (Fry et al. 1994, Su and Mulla 2002c) but repeated introduction would be essential to lasting effectiveness. Vacant niches may also occur in new ground pools created by irrigation, typically a rice field development. Colonization of invertebrate predators from

nearby sources may be earlier than supposed (Mogi et al. 1999), but if retarded under some situations, artificial introduction could help build-up of predator populations. Guppies and other larvivorous fish increased rapidly in a new dam lake constructed in West Timor, but they did not enter new irrigation canals immediately; new rice fields isolated from existing ones were virtually fish-free for 2 years, resulting in unusually high densities of larval anophelines in some fields (Mogi et al. unpublished data).

The strategy of maximizing effectiveness of indigenous predators can, in principle, be applied to both ground pools and natural containers. One option is to use selective pesticides to kill target mosquitoes with the least damage to local predators (Mulla 1990). Microbial insecticides and insect growth regulators are now widely used in this context but some damage to non-target organisms may not be avoidable in every instance (Niemi et al. 1999).

A more active option is to manage habitats so as to enable local predators to express their best abilities for mosquito control. This option has been tried for swamps and ponds, especially those created for flood control, waste water processing and/or nature conservation, in California (Batzer and Resh 1991, 1992; de Szalay et al. 1996, de Szalay and Resh 1997, 2000; Walton and Workman 1998, Thullen et al. 2002) and Australia (Russell 1999, Greenway et al. 2003). These studies demonstrate that increase in vegetation and structure heterogeneity generally increases diversity and abundance of macro invertebrates, including predators, and reduces mosquitoes, without obstructing the primary objectives. Vegetation heterogeneity can be increased by controlled mowing (de Szalay et al. 1996, de Szalay and Resh 1997, 2000), burning (de Szalay and Resh 1997), and flooding (de Szalay and Resh 2000). Structural heterogeneity can be increased by a combination of shallow and deep areas (Walton and Workman 1998, Greenway et al. 2003).

The leading concept in pest control in the late 20th century was integrated pest management (IPM). A more comprehensive concept proposed for management of rice lands is integrated biodiversity management (IBM) (Kiritani 2000). Rice lands are man-made multi-function wetlands supporting rich faunas (Kiritani 2000, Lawler 2001, Bambaradeniya et al. 2004). IBM serves to reconcile pest control with nature conservation. The target of management is biodiversity itself, including rice pests, their natural enemies and other terrestrial and aquatic animals of no economic significance (Kiritani 2000). An earlier advocate toward this direction was Heckman (1979) who studied biodiversity of rice fields in northern Thailand. He admired the traditional way of rice field management in that the rice field ecosystem is cultivated as a whole and several of its component species such as rice, fish and aquatic vegetables are harvested in a sustainable manner. Suppression of rice pest densities by diverse predators under no or least insecticide use has demonstrated the potential of IBM in rice lands in tropical Asia (Way and Heong 1994, Settle et al. 1996). Success of IBM is more difficult for mosquitoes in rice fields, because many important vectors use newly transplanted fields before both terrestrial and aquatic predators buildup their populations (Settle et al. 1996, Schoenly et al. 1998). Nevertheless, the IBM concept is essential, because rice fields as temporary wetlands sustain regional biodiversity (Lawler 2001). Temporary pools producing mosquitoes were formerly viewed as waste or useless, but now they have been re-evaluated as habitats with unique faunas including endangered species (Collinson et al. 1995, Williams 1997, Baber et al. 2004, Eitam et al. 2004). Development of IBM in various habitats could help mosquito control through higher and more stable activities of polyphagous predators. Inclusion of mosquito control into IBM could be cost-effective and could be optimized through coordination among various sectors.

Besides conceptual issues, success of mosquito control depends on availability of more tools for control. Study of predator ecology may yield cues for development of new mosquito control tools. For example, chemicals responsible for mosquito avoidance of predator habitats might lead to a new type of bio-degradable and safe repellents applicable to mosquito habitats. Toxins on spider webs and, if confirmed, from *Mesostoma* flatworms, might contribute to development of new type insecticides.

CONCLUSION

Many species of aquatic insect and other invertebrate predators have been noticed or investigated due to their potential as biological agents for mosquito control. Those predators play important roles in natural control of mosquitoes in ground pools and natural containers (phytotelmata). For those habitats with local predators, the ecologically sound strategy for mosquito control is to maximize their effectiveness through environmental management. In contrast, predators are usually absent or sparse in artificial containers in residential areas. For artificial containers and some other habitats lacking predators, artificial introduction of local predators can be an option in mosquito control.

Studies on ecosystems in small and often transient aquatic habitats are limited, compared to their diversity and ubiquity. This sharply contrasts with the extensive study on ecosystems in permanent aquatic habitats like lakes, ponds

and rivers. More studies on ecosystems and their component species in mosquito habitats will lead to effective use of biological agents in mosquito control as well as discovery of new candidates.

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A CRITICAL REVIEW OF ULTRALOW-VOLUME AEROSOLS OF INSECTICIDE APPLIED WITH VEHICLE-MOUNTED GENERATORS FOR ADULT MOSQUITO CONTROL[1]

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ABSTRACT. This review of ultralow-volume (ULV) ground aerosols for adult mosquito control includes discussion on application volume, aerosol generators, droplet size, meteorology, swath, dispersal speed, assay methods, insecticide efficacy, and nontarget effects. It summarizes the efficacy of ULV insecticidal aerosols against many important pest and disease-bearing species of mosquitoes in a wide range of locations and habitats in the United States and in some countries of Asia and the Americas. Fourteen conclusions were drawn from the review. 1) ULV ground aerosol applications of insecticide are as efficacious against adult mosquitoes as high- or low-volume aerosols. 2) ULV aerosols with an optimum droplet size spectrum can be produced by several types of nozzles including vortex, pneumatic, and rotary. Droplet size of a particular insecticide formulation is dependent primarily on nozzle air pressure or rotation speed and secondarily on insecticide flow rate. 3) Label flow rates of insecticide for ULV aerosol application can be delivered accurately during routine operations with speed-correlated metering systems within a calibrated speed range, usually not exceeding 20 mph. 4) The most economical and convenient method of droplet size determination for ULV aerosols of insecticide is the waved-slide technique. 5) The efficacy of ULV ground aerosols against adult mosquitoes is related to droplet size because it governs air transport and impingement. The optimum droplet size for mosquito adulticiding is 8–15 μm volume median diameter (VMD) on the basis of laboratory wind-tunnel tests and field research with caged mosquitoes. 6) In general, ULV aerosols should be applied following sunset when mosquitoes are active and meteorological conditions are favorable for achieving maximum levels of control. Application can be made during daytime hours when conditions permit, but rates may have to be increased. The critical meteorological factors are wind velocity and direction, temperature, and atmospheric stability and turbulence. 7) Maximum effective swaths are obtained with aerosols in the optimum VMD range during favorable meteorological conditions in open to moderately open terrain. The insecticide dosage must be increased in proportion to increased swath to maintain the same level of mosquito control. 8) Dispersal speed within a range of 2.5–20 mph is not a factor affecting efficacy if insecticide rate and optimum droplet size are maintained. 9) The results of caged mosquito assays are comparable with reductions in free-flying natural populations. 10) The field efficacies of mosquito adulticides applied as ULV ground aerosols are predictable from the results of laboratory wind-tunnel tests. 11) Results of field tests in open to moderately open terrain during favorable meteorological conditions indicated that ULV insecticidal aerosol application rates producing 90% or more control of *Anopheles, Culex,* and *Psorophora* spp. are below or ≈equal to maximum United States Environmental Protection Agency label rates. Against some *Aedes* spp., some pyrethroid insecticides must be synergized to produce 90% control at label rates. 12) Results of field tests in residential areas with moderate to dense vegetation and in citrus groves or other densely wooded areas showed that insecticide rates of ULV ground aerosols must be increased 2–3-fold to obtain 90% or more control of adult mosquitoes. However, the maximum rates on some insecticide labels would have to be increased to allow higher application rates. 13) Applications of ULV ground aerosols of insecticide in accordance with label directions following sunset do not pose a serious threat to humans, nontarget beneficial animals, or automotive paints. 14) Some aerosol generators operated at high RPM levels exceed the OSHA 8-h hearing hazard criteria of 90 dBA and may require hearing protectors for operators.

KEY WORDS Ultralow-volume, ULV, insecticide, adulticide, ground aerosol, mosquito, droplet size

INTRODUCTION

The recent review of ultralow-volume (ULV) aerial sprays of insecticide for mosquito control (Mount et al. 1996) provided a stimulus for a comparable review of ULV ground aerosols. No comprehensive review of ULV ground aerosols has been published previously, although Lofgren (1970, 1972) and Mount (1979, 1985) included ground aerosols in articles on ULV technology. ULV is the application of the minimum effective volume of an undiluted formulation of insecticide in liquid form as received from the manufacturer. The concentration of insecticide in an undiluted formulation may vary from only 2% for some of the pyrethroids to 85% or more for several of the liquid technical formulations of organophosphates. The application volume of an insecticide formulation is dependent on its liquid concentration and intrinsic toxicity to the target mosquito species. However, in cases where the applicator mixes the insecticide formulation with limited quantities of a solvent or carrier for various reasons, the application would be considered low volume (LV) because the minimum volume was not applied.

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

[2] Retired collaborator.

Mount et al. (1968) introduced ULV ground aerosols for adult mosquito control following successful ULV aerial spray applications by Knapp and Roberts (1965) and Glancey et al. (1965). For ULV ground aerosol application, Mount et al. (1968) modified a nonthermal aerosol generator (Curtis® 55,000, Curtis Dyna-Fog Ltd., Westfield, IN) that had been developed by the U.S. Army Engineers Research and Development Laboratories, Fort Belvoir, VA (Edmunds et al. 1958). Previously, Mount et al. (1966) indicated that nonthermal aerosols of insecticides diluted in fuel oil or water were comparable in efficacy with high-volume (HV) thermal aerosols of insecticide diluted in fuel oil, the standard atomization method at that time. These results, confirmed by Mount and Lofgren (1967), Taylor and Schoof (1968), and Mount et al. (1969b), demonstrated that diluents and atomization methods were not critical to mosquito control. After initial studies by Mount et al. (1968, 1970b), McNeill and Ludwig (1970), Mount and Pierce (1971), Taylor and Schoof (1971), and Rathburn and Boike (1972a), the ULV ground aerosol method was quickly advanced by the development of commercial ULV generators and the registration of technical and highly concentrated formulations of insecticide for ULV ground aerosol application by the United States Environmental Protection Agency (US-EPA). Within a few years, the ULV ground aerosol method was adopted by many mosquito control organizations throughout the United States and other countries of the world. ULV has been the worldwide standard ground aerosol method of mosquito adulticiding for more than 25 years because of the inherent advantages over HV aerosols. These advantages include lower cost because of the elimination of oil diluents and fuel required for thermal atomization, elimination of the dilution process, an increased effective payload, more rapid and timely application, and increased safety by elimination of dense fogs created by HV thermal atomization.

This review includes references on vehicle-mounted ULV ground aerosol applications of insecticide that were published through 1997 and is presented in chronological order within topical area. Major topics are volume, aerosol generators, droplet size, meteorology, swath, speed, assay methods, efficacy, and nontarget effects. Also, 14 conclusions and 11 summary tables based on the review are provided.

VOLUME

The very essence of the ULV method is minimum application volume. The application of an effective dose of insecticide, undiluted as received from the manufacturer, against target species of mosquitoes is ULV. If the insecticide is diluted by the applicator, then the application is LV or HV. In some cases, manufacturers offer a series of concentrations of the same insecticide, all labeled as ULV formulations. For example, synergized permethrin is labeled and marketed as ULV formulations of 1.5, 3, 3.98, 4, 10, 12, 30, and 31.28%. Only the 30 and 31.28% formulations are ULV, whereas the others are LV or HV. HV applications require HV equipment and are now seldom used by organized mosquito control in the United States. LV applications, where the manufacturer's insecticide formulation is less than maximum concentration or is diluted by the applicator in light mineral oils, refined soybean oil, heavy aromatic naphtha (HAN), or other carriers, are applied with ULV aerosol generators. Are these dilutions needed to maintain or increase the efficacy and swath of various insecticide formulations? The following review shows clearly that diluents and increased volume do not enhance insecticidal efficacy or extend swath. Instead, they show an inverse relationship between dilution and volume. As the percentage of active insecticide in a formulation is decreased, the application volume must be proportionally increased to maintain the same level of mosquito kill. Inert diluents do not kill mosquitoes. Diluents represent only an added cost for purchase and handling. The ULV method was developed to avoid these unnecessary costs.

ULV versus HV: Results by Mount et al. (1968) indicated that the insecticidal efficacy of ground aerosols is unrelated to application volume. In their study, flow rates of 0.36 and 0.26 fl. oz./min of undiluted 95% malathion and 85% naled, respectively, applied at 5 mph provided caged mosquito kills over swaths of 600 ft. that equaled or exceeded kills with a HV rate of 85 fl. oz./min of equal doses of insecticide diluted in fuel oil and dispersed at 5 mph. Furthermore, in tests with natural populations of *Aedes taeniorhynchus* (Wied.) in citrus groves, they obtained better control with 0.51 fl. oz./min of 85% naled than with 85 fl. oz./min of naled diluted in fuel oil dispersed at twice the dose of naled as the ULV application. The initial results with caged mosquitoes by Mount et al. (1968) were confirmed by Mount et al. (1970b) and Rathburn and Boike (1972a), who demonstrated that flow rates of 1.43 and 1.11 fl. oz./min of 95% malathion were equal or better in efficacy over 600-ft. swaths than a HV rate of 85 fl. oz./min of the same doses of malathion diluted in fuel oil.

ULV versus LV: Against *Ae. taeniorhynchus,* Mount et al. (1972) reported equal results with ULV aerosols of 1.37 fl. oz./min of 85% naled and LV aerosols of 13.70 fl. oz./min of 8.5% naled diluted in HAN or soybean oil. However, the HAN formulation had to be atomized at a lower nozzle pressure than the other formulations to achieve an optimum droplet spectrum. Also, Rathburn et al. (1981, 1986) did not increase the efficacy of naled against *Aedes* and *Culex* spp. with formulations of 3–10% Dibrom®$_{14}$ in HAN or various oils applied at 10–21 fl. oz./min compared with results with undiluted Dibrom$_{14}$ (Valent, Walnut Creek, CA). In

tests with chlorpyrifos applied at 10 mph over 1,500-ft. swaths against caged adult *Culex pipiens* Linn., Husted et al. (1975) reported slightly higher overall kills with 1.33–1.7 fl. oz./min of a 6-lb. AI/gal ULV formulation than with 7.8–9.0 fl. oz./min of a 1-lb. AI/gal LV formulation. Furthermore, Rathburn and Boike (1981) indicated similar kills of *Culex* spp. with flow rates of 2.1 fl. oz./min of 91% malathion and 3.2–4.3 fl. oz./min of 91% malathion mixed with HAN at a 7:5 ratio. However, Bunner et al. (1987) obtained higher mortalities of *Ae. taeniorhynchus* with a malathion:HAN mixture (1:4) than with undiluted 91% malathion. In their tests, better results with LV aerosols could have resulted from a difference in droplet spectrum rather than increased volume or increased toxicity. Finally, in tests at different times and locations with synergized resmethrin against caged *Anopheles quadrimaculatus* Say, Mount et al. (1974c) and Sandoski et al. (1983) reported results with flow rates of 2.57 and 0.9 fl. oz./min that were similar to those obtained with 12 fl. oz./min of comparable doses of insecticide and synergist diluted 1:8 in various oils (Weathersbee et al. 1991, Groves et al. 1994).

ULV flow rates: Although not designed as studies of application volume, many tests with caged adult mosquitoes have indicated 90% or more kill with flow rates of undiluted insecticide at ≈1 fl. oz./min or less dispersed at 10 mph. These tests include flow rates (fl. oz./min) of 0.84 of 91% malathion (Roberts 1983); 0.95, 0.97, and 0.79 of 93% fenthion (Mount et al. 1970b, 1978a; Mount and Pierce 1971); 1.01 of 85% naled (Mount et al. 1970b); 1.05 of 6 lb. AI/gal of chlorpyrifos (Rathburn and Boike 1975); 0.84 and 0.80 of 40% resmethrin (Rathburn and Boike 1972b, Sandoski et al. 1983); 0.90 of 18% resmethrin plus 54% piperonyl butoxide (Sandoski et al. 1983); and 0.79 of 3.6 lb. AI/gal permethrin (Kline et al. 1986). Moreover, in studies designed to determine minimum effective dose, flow rates of <0.5 fl. oz./min of various insecticides provided 50–90% mosquito kill because of low doses rather than insufficient application volume (Mount and Pierce 1971, 1972b; Mount et al. 1968, 1974c, 1978a). Flow rates of <0.5 fl. oz./min can be metered accurately with most ULV aerosol generators, which eliminates the need for dilution with most insecticide formulations unless specified by the label. Labels that require dilution should be modified to allow ULV application.

AEROSOL GENERATORS

Many improvements in ULV aerosol generators have been made since the modifications of a military nonthermal generator by Mount et al. (1968), who pointed out that other types of equipment could be used to produce ULV aerosols. Mount et al. (1970b) adapted an aerosol nozzle developed by Lowndes Engineering Co., Inc. (Leco®, Valdosta, GA) to a Leco 120 thermal aerosol generator and a Curtis 55,000 nonthermal military generator. Maximum nozzle pressure with the modified Leco 120 was only 3.5 psi, which was adequate for 3 fl. oz./min of 95% malathion volume median diameter [VMD] = 15 μm) but not for 6 fl. oz./min (VMD = 20 μm). McNeill and Ludwig (1970) also used a ULV conversion on a Leco 120 generator for application of 1.065–2.13 fl. oz./min of technical malathion. Anderson and Schulte (1970) described the practical aspects of converting thermal aerosol generators to ULV. Their conversion consisted of removing all parts from a Leco 120 or Tifa® 100E thermal machine (Tifa, Ltd., Millington, NJ) except the engine and blower and then installing an insecticide metering system and a Leco ULV nozzle. The insecticide metering system consisted of a stainless steel tank pressurized by the blower, chemical-resistant tubing, flow meter, needle valve, and solenoid valve for remote operation. With thermal generators modified for ULV application, Anderson and Schulte (1970) reported 2–3 times coverage capability per generator at less than 33% of the cost of previous operations with HV thermal aerosol generators.

In recent years, 6 aerosol generators, including the Leco 1600 (Robinson and Ruff 1990), London Fog® 18–20 ULV (Robinson and Ruff 1991a; London Fog, Inc., Long Lake, MN), Curtis Dyna-Fog® Maxi-Pro 4 ULV (Robinson and Ruff 1991b; Curtis Dyna-Fog, Ltd., Westfield, IN), Conner Engineering Bison® (Robinson and Ruff 1992; Clarke Engineering Technologies, Inc., Roselle, IL), Beecomist® Systems Pro-Mist 25HD ULV (Robinson et al. 1993; Beecomist Systems, Telford, PA), and VecTec® Grizzly (Robinson 1994; Clarke Engineering Technologies, Inc.), have been evaluated at the Pasco County Mosquito Control District, Odessa, FL (Collaborating Center on Testing and Evaluation of Pesticide Application Equipment for the World Health Organization). These comprehensive evaluations provide data on the manufacturer, price, chassis, dimensions, nozzle, blower/compressor, engine, fuel capacity and consumption, instrumentation, remote control, options, gauge accuracy, flow control accuracy, noise levels, and droplet spectrum.

Nozzles: Three types of nozzle systems have been used to generate ULV aerosols. These are low-air-pressure and high-air-volume vortex, high-air-pressure and low-air-volume pneumatic, and rotary sleeve. In this review, generators equipped with vortex nozzles were the Curtis Dynafog Cyclotronic and Maxi-Pro 4 ULV, Leco HD-ULV and 1600, London Fog 18–20 ULV, Micro-Gen® MS2–15 and LS2–15 (Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO), Micro-Mist®, Tifa® 100-E-ULV, and VecTec Grizzly. Those using pneumatic nozzles were the Buffalo Turbine® Sonic, Conner Engineering Bison, and London Aire®

XW. The Beecomist Systems Pro-Mist 25 HD, Cardinal 150, and Whisper-Mist 10 used rotary-sleeve nozzles.

Insecticide metering systems: The widespread conversion from thermal aerosol generators to ULV generators by organized mosquito control stimulated interest in development of improved ULV insecticide metering systems. Metering systems were needed that could deliver accurate flow rates under a wide range of operating conditions. With a needle valve and flowmeter system, Rathburn and Boike (1972a) and Fultz et al. (1972) discovered the need for a temperature-corrected calibration curve for dispersing 95% malathion. Their observations indicated that an adjustment in the flowmeter setting was required with each 2°F variation in temperature. To eliminate the need for temperature correction and to improve flow rate accuracy, manufacturers of aerosol generators used positive displacement pumps with electronic speed control to vary flow rate. Once calibrated for a particular insecticide, positive displacement pumps would deliver constant flow rates, as shown by Fleetwood et al. (1980). By the end of the 1970s, commercial metering systems featuring advanced electronic technology had been developed that provided speed-correlated flow control (Street 1980). Also, a simpler mechanical method involving a speed-monitoring device was developed by James Robinson, Pasco County Mosquito Control District, Odessa, Florida, and described by Street (1980). Speed-correlated flow control systems are now standard technology for ULV aerosol generators and are capable of delivering flow rates within ≈6% of a target label rate during routine operations (Dame and Curtis 1990).

DROPLET SIZE

Prior to 1968, there was limited consideration of aerosol droplet size for mosquito control compared with the current emphasis. With thermal aerosols, a relationship between generator heater temperature, insecticide formulation flow rate, and mosquito kill had been determined by empirical methods. Recently, Brown et al. (1993b) collected droplets of no. 2 fuel dispersed from a Leco 120D thermal aerosol generator on hand-waved Teflon®-coated glass microscope slides (DuPont, Wilmington, DE) for determination of droplet size. Their results indicated VMDs of 15–18 μm for a flow rate of 29 gph and heater temperature of 750°F, which would be comparable with 40 gph and 850°F used by Mount et al. (1968) to generate HV thermal aerosols for comparison with ULV aerosols. Thus, the droplet size estimates by Brown et al. (1993b) suggested that the droplet spectra of thermal aerosols were similar to those for ULV aerosols. The VMD is a droplet diameter where 50% of the aerosol volume is in larger droplets and 50% is in smaller droplets. VMD is used in reference to both volume median diameter and mass median diameter in this review.

Measurement methods: The primary objective of droplet size measurement is to estimate the initial droplet spectrum as dispersed from the aerosol generator. This measurement is necessary to relate the droplet spectrum of the total aerosol volume to mosquito kill efficiency. Droplet size number distribution, by comparison, is relatively unimportant. For example, Mount and Pierce (1972a) reported that 83–94% of malathion droplets dispersed from a Leco HD-ULV generator operated at 4 psi were less than 5 μm in diameter. However, these small droplets represented only 7% of the total volume of malathion dispersed. In operational programs, droplet size measurement is needed to optimize mosquito kill efficiency and to meet label requirements, as emphasized by Walcher (1993). During the development of ULV ground aerosols, Mount et al. (1968) used methods reported by Yeomans (1949) for the initial droplet size estimates of technical malathion aerosols. Rathburn (1970) provided a comprehensive review of methods for assessing the droplet size of insecticidal sprays and aerosols, including Yeomans's method. This method, with modifications, is still in use because it is rapid, convenient, and economical. These modifications include replacement of silicone glass slide coating with Teflon for further reduction in droplet spread (Anderson and Schulte 1971), use of a "direct measurement method" and refinement of the focal-length method to determine droplet spread (Anderson and Schulte 1971, Mount and Pierce 1972a, Dukes et al. 1993), variation in slide-wave technique (Beidler 1975, Peterson et al. 1978, Carroll and Bourg 1979, Brown et al. 1990, Wilhide and Daniel 1995), and computer programs for rapid and convenient calculation of droplet size parameters (West and Cashman 1980, Sofield and Kent 1984, Boobar et al. 1986). Two additional methods of droplet size collection discussed by Rathburn (1970) include settling and impaction. Mount and Pierce (1972a) used these methods to confirm the accuracy of the slide-wave method described by Yeomans (1949).

Haile et al. (1978) developed a method for automatic measurement of the droplet size of insecticidal aerosols with a Coulter Counter® (Beckman Coulter, Inc., Fullerton, CA). Samples of technical malathion aerosols introduced into settlement chambers were collected in a liquid medium placed on the floor of the chamber. Automatic droplet count and size analysis was then accomplished by electronic current path interruption when the liquid containing the droplets passed through a small aperture. Concurrent sampling of droplets of malathion, fenthion, and Klearol® (white mineral oil) aerosols for VMD determination with the Coulter Counter method and standard microscope measurement showed similar results. However, Coulter estimates were less variable than microscope esti-

mates because of the large difference in droplets measured per sample (50,000–100,000 in 90 sec for Coulter versus only 300 in 30 min or more for microscope). The disadvantages of the Coulter method were the high equipment cost and demanding protocol required for collecting, handling, and reading liquid samples. Nevertheless, results from the Coulter study confirmed the accuracy of the standard waved-slide method that is currently used to estimate the VMD of ULV aerosols.

A hot-wire instrument, the Army Insecticide Measuring System (AIMS), has been used operationally (Swartzell 1991) and compared with other methods (Brown et al. 1993c). Brown et al. showed that the hot-wire method produced VMDs that were similar to those obtained from Teflon-coated slides waved through the aerosol cloud or placed in a chamber used for aerosol settlement. However, they also determined that the hot-wire method was sensitive to the aerosol generator air blast and that a preferred sampling distance must be determined for each model of generator to obtain accurate data. Advantages of using AIMS were relative ease of use, large droplet sample (10,000 in 100 sec), and immediate analysis of results in the field.

Phillips and Kutzner (1994) used a Malvern® model 2000 laser droplet analyzer to compare the droplet sizes of malathion and permethrin aerosols dispersed from a Leco HD aerosol generator and a Beecomist Systems Pro-Mist 25 HD rotary atomizer. With a Leco HD dispersing 4.3 fl. oz./min of 95% malathion, their VMD estimate of 11 μm at 4.5 psi nozzle pressure was slightly less than estimates of 13–15 μm reported previously by Beidler (1975), Mount et al. (1975a, 1975b), Mount and Pierce (1976), and Rathburn and Boike (1977). The laser analyzer showed that most of the aerosol volume was in droplets of 5–25 μm diameter, which is comparable with 67–73% in the same range obtained by impaction and settling methods (Mount and Pierce 1972a). The VMD estimate for the same flow rate of 95% malathion dispersed by the Pro-Mist 25 HD generator was 17 μm, with most of the volume in the 5–25-μm-diameter range. The VMD estimates from the laser analyzer for 6 fl. oz./min of 4% permethrin plus 12% piperonyl butoxide were 9 and 20 μm for the Leco HD-ULV (4.5 psi) and the Pro-Mist 25 HD rotary atomizer, respectively. Previously, Mount et al. (1978a) estimated similar VMDs of 8 μm for a 2-lb. AI/gal formulation of permethrin dispersed by a Leco HD-ULV operated at 4 psi (waved-slide and settlement chamber methods).

Optimum droplet size: A critical factor in the successful development of ULV ground aerosols was droplet size. A review of previous research on droplet size (Mount 1970) suggested that the optimum droplet size for outdoor adult mosquito control was 11–20 μm. Thus, Mount et al. (1968, 1970b) varied droplet size by changes in nozzle air pressure and insecticide flow rate with ULV aerosol generators to study the relationship between droplet size and mosquito kill under field conditions (Table 1). Their results with three different dosages of 95% malathion indicated that aerosols of 8–15 μm VMD were consistently more effective against caged adult female *Ae. taeniorhynchus* than those of 15–28 μm VMD.

Table 1. Kill of caged adult female mosquitoes with ultralow-volume aerosols of 95% malathion as influenced by dose, droplet size, and downwind distance (after Mount et al. 1968, 1970b; Haile et al. 1982)

Dose (fl. oz./mi.)[1]	VMD (μm)[2]	Percentage kill at indicated feet downwind			
		150 ft.	300 ft.	600 ft.	Mean
		Aedes taeniorhynchus			
4.3	15–17	34	28	13	25
4.3	8–10	53	38	33	41
12.0	30–39	61	48	24	44
8.5–12.0	16–24	92	74	51	72
8.5–12.0	8–15	92	91	76	86
12.0	5	68	70	57	65
17.0	16–28	93	90	91	91
17.0	10–14	100	100	98	99+
		Anopheles quadrimaculatus			
12.0	30–39	67	48	29	48
12.0	24	95	68	60	74
12.0	8–15	85	73	63	74
12.0	5	87	69	56	71

[1] 1 fl. oz./mi. = 18.5 ml/km.
[2] Volume median diameter (VMD) values in Mount et al. (1968, 1970b) were multiplied by 1.25 to reflect a spread factor of 0.5 instead of 0.4 for silicone-treated slides.
[3] 1 ft. = 0.3048 m.

In a laboratory study, Weidhaas et al. (1970) determined that the minimum lethal dose (LD_{100}) of technical malathion for *Ae. taeniorhynchus* adult female mosquitoes was contained in a 25-μm-diameter droplet. They also extrapolated from malathion to determine that 20- and 17.5-μm-diameter droplets of 85% naled and 93% fenthion, respectively, would also contain LD_{100}s. The results of this study suggested that optimum droplet sizes for aerosols of these insecticides are likely not greater than the size containing the LD_{100} because larger sizes would contain more insecticide than necessary to kill a single mosquito.

In another study, Lofgren et al. (1973) used a scanning electron microscope to observe aerosol droplets impinged on adult mosquitoes. In field tests, caged adult female *Ae. taeniorhynchus* were exposed to ULV aerosols of soybean oil (used to simulate malathion) with a VMD of 19 μm. Results indicated that 100% of the total mass impinged on mosquito wings was in droplets of 2–16 μm diameter. Also, all of 39 droplets observed on the wings of free-flying female *Ae. taeniorhynchus* mosquitoes that had been exposed to ULV aerosols in the field were 1–8 μm diameter. In laboratory experiments, free-flying adult female *Ae. taenio-*

rhynchus were exposed to aerosols of soybean oil with a VMD of 7.7 μm that were produced with a Babington nebulizer (Litt and Swift 1972). Results showed that 99% of the total mass impinged on mosquito wings was in droplets of 2–10 μm diameter.

The results of the initial studies by Mount et al. (1968, 1970b) were confirmed by Haile et al. (1982) in both laboratory and field tests with caged mosquitoes. Analysis of malathion droplets produced from a Berglund-Liu Monodisperse Aerosol Generator in 18 different uniform sizes in a range of 2.8–32.8 μm and dispersed in a wind tunnel against *Ae. taeniorhynchus* indicated that the optimum droplet size range was 10–15 μm diameter. Also, insecticidal efficiency decreased rapidly for sizes smaller than 5 μm diameter and larger than 25 μm diameter. Field tests with 12 fl. oz./mi. of 95% malathion against *Ae. taeniorhynchus* and *An. quadrimaculatus* indicated 82% mosquito kill with 10- and 15-μm VMD aerosols compared with 33, 67, and 72% kill for 39-, 5-, and 24-μm VMD aerosols, respectively. These results are combined with those by Mount et al. (1968, 1970b) in Table 1.

Results by Rathburn and Dukes (1989) suggested that the initial droplet size (≈15 μm VMD) decreased ≈50% with droplets collected at 300 ft. downwind during winds of 2–3 mph (6.8 and 7.5 μm VMD in vegetated and open areas, respectively). A similar study by Brown et al. (1993a) indicated a decrease of ≈33% (27 to 18 μm VMD) in size of droplets collected at 300–400 ft. downwind from a Beecomist Systems Whisper-Mist 10 aerosol generator dispersing 4 fl. oz./min of 91% malathion during unspecified winds. Data from these 2 droplet collection studies suggest that the optimum droplet size for malathion aerosols is less than 15–27 μm VMD because the larger droplets in these applications remained airborne for less than 300 ft.

Recently, Curtis and Beidler (1996) studied the effect of droplet size of ULV permethrin aerosols on caged *Ae. taeniorhynchus* placed at distances of 100–500 ft. downwind in a mature citrus grove consisting of moderately dense vegetation. Their results indicated that, at equal doses, aerosols with a 15-μm VMD produced higher mosquito kills than aerosols with 7- and 26-μm VMDs. The 7-μm VMD aerosols gave consistently lower percentage kills at all distances than the other aerosols. Although the 26-μm VMD aerosols were about equal to the 15-μm VMD aerosols at 100–300 ft., they produced lower kills at 400 and 500 ft.

Droplet size estimates: Estimates of VMD for malathion are shown in Table 2, whereas those for chlorpyrifos, fenthion, naled, permethrin and propoxur are listed in Table 3. Only portions of the total data in two studies with malathion (Mount et al. 1968, Dukes et al. 1990) that included several combinations of nozzle pressures and flow rates are shown in Table 2. Results of the initial studies by Mount et al. (1968, 1970b) showed an inverse relationship between droplet size and nozzle pressure. Estimates of VMD consistently decreased as nozzle pressure increased. For example, at a flow rate of 1.5 fl. oz./min of 95% malathion, VMD decreased 64% (28 to 10 μm) as nozzle pressure increased 375% (1.6 to 6 psi). Their results also indicated a direct relationship between droplet size and insecticide flow rate, although the flow rate effect was less than that of nozzle pressure. At a constant nozzle pressure of 3.5 psi with the Leco ULV nozzle, the VMD increased only 33% (15 to 20 μm) as the flow rate of malathion was increased 400% (1.5 to 6 fl. oz./min). These relationships between droplet size of malathion aerosols and nozzle pressure or flow rate were confirmed by the results of Mount and Pierce (1972b), Peterson et al. (1976), Rathburn and Boike (1977), Haile et al. (1982), and Dukes et al. (1990). These relationships were also demonstrated for chlorpyrifos, naled, and permethrin (Mount and Pierce 1972a, 1972b; Curtis and Beidler 1996).

Estimates of VMD shown in Tables 2 and 3 indicated that all of the aerosol generators included in this review can, with appropriate nozzle pressure or rotational speed and flow rate combinations, atomize malathion and other insecticides to meet label requirements and achieve maximum or near maximum efficiency in killing adult mosquitoes. Although VMD estimates in Table 2 indicated that excessive atomization is not likely to occur with technical malathion at flow rates of 3 fl. oz./min or more, studies with less viscous or more volatile insecticide formulations indicated that overatomization is a possibility. For example, Mount and Pierce (1972b) estimated a 5-μm VMD and obtained unsatisfactory mosquito kill with naled diluted in heavy aromatic naphtha.

METEOROLOGY

For ULV ground aerosols of insecticide against adult mosquitoes, the critical meteorological parameters are wind velocity and direction, temperature, and atmospheric stability and turbulence. Although most of the research reviewed in this paper does not directly relate meteorology to mosquito control, general guidelines can be interpreted. Also, the results from one comprehensive study (Schatmeyer and Urone 1973) correlating meteorological parameters with mosquito kill are discussed. These investigators used a portable meteorological station that consisted of a trailer-mounted tower and electronic instrumentation housed in a van-type vehicle. The tower design was patterned after a similar unit used by Rathburn and Miserocchi (1969). The electronic equipment was arranged to continuously record meteorological measurements for 6 entire evenings of 3–4 aerosol runs per evening in a residential area of Gainesville, FL.

Wind velocity and direction: Some horizontal wind velocity is required to drift an insecticidal

Table 2. Volume median diameters (VMD) of ultralow-volume (ULV) ground aerosols of 91–96% malathion (Cythion®, Fyfanon®).

Aerosol generator[1]	Nozzle (psi)[2]	Fl. oz./min[3]	Sizing method[4]	VMD (μm)[5]	Reference
Curtis® 55,000[6]	1.6, 2.6, 4, 6	1.5	WS	28, 18, 14, 10[7]	Mount et al. (1968)
Leco® ULV	3.5	1.5, 3, 6	WS	15, 16, 20[7]	Mount et al. (1970b)
Leco HD-ULV	4	3.0	WS, SC, CI	18, 16, 17	Mount and Pierce (1972a)
Leco HD-ULV	2.5, 4, 5	3.0	WS	21, 18, 14	Mount and Pierce (1972b)
BTS, M-MS, L-HD	110, 4, 4	3.0	WS	25, 16, 15	Mount et al. (1975a)
M-LS, L-HD	6, 6	4.3	WS	12, 11	Mount et al. (1975c)
Leco HD-ULV	4.5	4.3	WS	14–15	Beidler (1975)
LA-XW,-L-HD	90, 4	4.3	WS	14, 15	Mount and Pierce (1976)
Leco HD-ULV	4.5, 5, 6	4.0	WS	17, 16, 14	Peterson et al. (1976)
BTS, LA-XW, M-LS	100, 90, 4.5	4.3	WS	14, 11, 12	Rathburn and Boike (1977)
MM, T-100, L-HD	65, 4, 4	4.3	WS	14, 17, 14	Rathburn and Boike (1977)
Leco HD-ULV	4	4.3	WS, Coulter Counter®	17–22, 17–27	Haile et al. (1978)
Leco HD-ULV	4	4.3	WS	11–18	Peterson et al. (1978)
Leco HD-ULV	3.5	4.0	WS, SC	21–25, 19–26	Carroll and Bourg (1979)
Leco HD-ULV	1, 3, 5, 7.5	1.0	Coulter Counter	30, 15, 10, 8	Haile et al. (1982)
London Aire® XW	90	1.0	Coulter Counter	5	Haile et al. (1982)
CDC, L-HD, L-HD-2	6	4.3	WS	13, 15, 14	Dukes et al. (1990)
CDC, L-HD, L-HD-2	7	8.6	WS	14, 16, 16	Dukes et al. (1990)
Leco 1600	6	4.3, 8.6	WS	15, 17	Robinson and Ruff (1990)
London Aire 18-20	6	4.3, 8.6	WS	16, 18	Robinson and Ruff (1991a)
Dyna-Fog® Maxi-Pro 4	6	4.3, 8.6	WS	15, 17	Robinson and Ruff (1991b)
Leco MD-ULV	8	6.0	WS	14–24	Perich et al. (1992)
Conners Engr. Bison®	90	4.3, 6.5	WS	16, 19	Robinson and Ruff (1992)
Leco HD-ULV	5	8.6	WS, AIMS	16, 16	Brown et al. (1993c)
Pro-Mist® 25HD ULV	—[8]	4.3, 6.5, 8.6	WS	15, 17, 18	Robinson et al. (1993)
L-HD, PM-25	4.5, —[8]	4.3	Laser analyzer	11, 17	Phillips and Kutzner (1994)
VecTec® Grizzly ULV	7.3–7.4	4.3, 8.6	WS	16, 17	Robinson (1994)

[1] L-HD = Leco® HD-ULV; L-HD-2 = Leco HD-ULV with 2 MD nozzles; BTS = Buffalo Turbine® Sonic; M-MS = Micro-Gen® MS2-15; M-LS = Micro-Gen® LS2-15; LA-XW = London Aire® XW; MM = Micro Mist®; T-100 = Tifa® 100-E-ULV; CDC = Curtis Dynafog® Cyclotronic; PM-25 = Beecomist® Systems Pro-Mist 25.

[2] Nozzle pressures for respective aerosol generators (1 psi = 6.894757 kPa).

[3] Flow rates for respective nozzle pressures and aerosol generators (1 fl. oz./min = 29.7 ml/min).

[4] Sizing methods for respective flow rates, nozzle pressures and aerosol generators. WS = waved slide; SC = settlement chamber; CI = cascade impactor; AIMS = Army Insecticide Measuring System.

[5] VMDs for respective sizing methods, flow rates, nozzle pressures, and aerosol generators.

[6] Modified for ULV.

[7] VMD values in references were multiplied by 1.25 to reflect a spread factor of 0.5 instead of 0.4 for silicone-coated slides.

[8] Not applicable because atomization was achieved by rotation (30,000+ RMP) instead of pressure.

Table 3. Volume median diameters (VMD) of ultralow-volume (ULV) ground aerosols of chlorpyrifos, fenthion, naled, permethrin, and propoxur.

Aerosol generator	Nozzle (psi)[1]	Fl. oz./min[2]	Sizing method	VMD (μm)[3]	Reference
			Chlorpyrifos, 6 lb. AI/gal (Dursban®)		
Leco® HD-ULV	1.5, 4	3.2	Waved slide	17, 10	Mount and Pierce (1972a)
Leco HD-ULV	1.5, 4	3.2	Settlement chamber	18, 10	Mount and Pierce (1972a)
Leco HD-ULV	1.5, 4	1.6	Waved slide	15, 9	Mount and Pierce (1972b)
Leco HD-ULV	1.5, 4	3.2	Waved slide	17, 10	Mount and Pierce (1972b)
Military generator[4]	3.5	1.3	Settlement chamber	9	Husted et al. (1975)
Leco HD-ULV	4	1.6	Waved slide	9	Mount et al. (1975c)
			Fenthion, 93% (Baytex®)		
Leco HD-ULV	4	2	Waved slide	10	Mount et al. (1975c)
Leco HD-ULV	4	1	Waved slide, Coulter Counter®	13, 13	Haile et al. (1978)
			Naled, 8.5% in heavy aromatic naphtha (Dibrom®)		
Leco HD-ULV	1.5, 4	7	Waved slide	15, 5	Mount and Pierce (1972b)
			Permethrin, 2 lb. AI/gal		
Leco HD-ULV	4	2	Waved slide, settlement chamber	8, 8	Mount et al. (1978a)
			Permethrin plus piperonyl butoxide, 4% + 12%		
Leco HD-ULV	4.5	6	Lazer analyzer	9	Phillips and Kutzner (1994)
Pro-Mist® 25	—[5]	6	Laser analyzer	20	Phillips and Kutzner (1994)
			Permethrin, 57% (Punt®)		
Dynafog® Cyclotronic	4.5, 3, 0.5	0.8	Waved slide	7, 15, 26	Curtis and Beidler (1996)
			Permethrin plus piperonyl butoxide, 31% + 66% (Permanone®)		
Leco 1600	3.2	9.4	Waved slide	16	Meisch et al. (1997)
			Propoxur, 1 lb. AI/gal (Baygon®)		
Leco HD-ULV	4	11.5	Waved slide	14	Mount et al. (1975c)
			Propoxur, 1.67 lb. AI/gal (Baygon®)		
Leco HD-ULV	4	9	Waved slide, settlement chamber	9, 9	Mount et al. (1978a)

[1] 1 psi = 6.894757 kPa.
[2] 1 fl. oz./min = 29.7 ml/min.
[3] VMDs for respective sizing methods, flow rates, and nozzle pressures.
[4] Modified for ULV.
[5] Not applicable because atomization was achieved by rotation (30,000+ rpm) instead of pressure.

aerosol cloud across the target area, typically 1 or 2 city blocks in width. The wind velocities indicated for all but 1 of the field efficacy studies summarized herein were <1–11 mph with an overall mean of ≈3.5 mph. Thus, wind velocities of 1–7 mph, with gusts not exceeding 11 mph, are likely the most suitable for aerosol applications against adult mosquitoes. Against caged *Ae. taeniorhynchus* and *Culex nigripalpus* Theobald, Rathburn and Boike (1972b) obtained slightly higher kill during wind velocities of 6–11 mph (78%) than 1–6 mph (68%) when mosquitoes were exposed to aerosols of 95% malathion and 3.3 lb. AI/gal resmethrin. From 21 separate aerosol runs during 6 evenings, Schatmeyer and Urone (1973) constructed a statistical linear model that indicated that mosquito kill was directly related to wind velocity. Even though their study included wind velocities of only 0.4–2.5 mph, the model predicted improved mosquito kill with increased wind velocity to ≈4 mph. Schatmeyer and Urone also speculated that wind velocities greater than 4 mph might be used to assist aerosol penetration into vegetated areas but only at short range. Because of a limited wind velocity range (<1–7 mph), Curtis and Mason (1988) were unable to demonstrate any statistically significant relationship between wind velocity and kill of caged adult female *Ae. taeniorhynchus* in a Florida citrus grove.

Wind direction data predict the direction of aerosol cloud drift. In experimental applications, wind direction should be parallel with rows of caged mosquito or observation stations and perpendicular to the aerosol generator line of travel. Floore et al. (1991) stated that their tests were not done when wind direction varied >45° from perpendicular to swath direction. Although not stated by other investigators, this procedure has been followed in most ULV ground aerosol studies. Other techniques that have been used to account for deviations in wind direction include calculation of actual downwind distance (Schatmeyer and Urone 1973) and statistical correction in mosquito kill for increased exposure distance (Curtis and Mason 1988, Curtis and Beidler 1996). For operational applications in large urban and suburban areas with extensive road networks, complete coverage can be obtained regardless of wind direction unless wind direction changes frequently during application. Frequent wind direction shifts during an application can cause incomplete coverage and require retreatment.

Temperature: Ambient temperature is important because it influences mosquito activity, but it may or may not influence the efficacy of some insecticides. Also, the vertical temperature gradient is 1 factor that determines atmospheric stability, which is discussed in the next section. Ambient temperatures reported for field efficacy studies included herein were 63–89°F with a mean of ≈79°F. Low temperatures can reduce the effectiveness of insecticides, as indicated by Stevens and Stroud (1967), who reported possible recovery of adult *Aedes stimulans* (Walker) in 12 h following an application of propoxur spray at ≈60°F in Michigan. However, Mount et al. (1969a) obtained satisfactory control of *Aedes* spp. with aerial sprays of malathion during ambient temperatures of <60°F in subarctic Alaska, where mosquitoes are apparently adapted to host-seeking activity during relatively low temperatures as compared with mosquito species in temperate and tropical climates. In Michigan, Knepper (1988) showed no correlation between temperature over a wide range (54–90°F) and percentage kill of *Cx. pipiens* and *Culex restuans* Theobald with a mixture of malathion, resmethrin and HAN. Furthermore, Curtis and Mason (1988) showed no temperature effect within a range of 74–89°F with applications of naled against caged *Ae. taeniorhynchus* in a Florida citrus grove.

Atmospheric stability and turbulence: Atmospheric stability and turbulence are important factors that influence aerosol cloud diffusion across the target swath. Factors that determine atmospheric stability are wind velocity, temperature gradient, and time of day. The relatively stable air associated with evening (≈6–11 p.m.) is generally considered the most suitable for aerosol applications. Of the field efficacy studies in this review that indicated application times, 85 and 15% were accomplished with evening and morning applications, respectively. Aerosol applications in the evening are usually more efficacious than those made in the morning because of more favorable meteorological conditions. For example, with aerosols of 95% malathion against *Ae. taeniorhynchus,* Mount and Pierce (1976) obtained a 90% effective rate of 0.162 lb. AI/acre with morning applications, whereas previous evening applications indicated 90% effective rates of 0.025–0.076 lb. AI/acre (Mount and Pierce 1971, 1972b; Mount et al. 1974c, 1975b, 1975c). Nevertheless, Mount and Pierce (1974) obtained more satisfactory daytime control of *Ae. taeniorhynchus* in small residential areas in the Florida Keys with morning aerosol applications of naled than with evening applications because of rapid and heavy mosquito reinfestation.

Stability ratios have been used as a measure of suitable meteorological conditions for wide-swath (660–5,280 ft.) aerosol applications against mosquitoes in California pastures. Womeldorf and Mount (1977), Miller et al. (1982) and Townzen et al. (1987) calculated stability ratios from a formula adapted from Haugen et al. (1961) as follows:

$$\text{Stability ratio} = \frac{t_2 - t_1 \times 10^5}{\bar{u}^2}$$

where

t_2 = temperature (°C at 10 m)

t_1 = temperature (°C at 3 m)

$\bar{u}$ = average wind velocity (cm/sec).

Table 4. Kill of caged adult female *Aedes taeniorhynchus* with ground aerosols of 18 fl. oz./mi. of 95% malathion as influenced by wind velocity, stability ratio, distance, and elevation, Gainesville, FL (after Schatmeyer and Urone 1973).

			Percentage 18-h kill at indicated feet downwind and (feet) elevation					
			150 ft.	300 ft.			500 ft.	Mean
Evening[1]	Wind velocity[2] (mph)	Stability ratio[3] (3–98 ft.)	(3 ft.)	(3 ft.)	(30 ft.)	(50–100 ft.)	(3 ft.)	(3 ft.)
1	1.4	0.5	44	23	32	—	7	25
2	1.2	−2.9	56	45	59	—	28	43
3	2.0	0.9	58	58	81	—	20	45
4	1.5	4.1	42	34	85	89	20	32
5	0.6	177.0	89	76	71	3	59	75
6	2.0	−1.1	81	47	92	84	57	62

[1] Each evening consisted of 3–4 runs of 3 fl. oz./min of 95% malathion dispersed with a Leco HD-ULV aerosol generator operated at 4 psi and 10 mph (aerosol applications by G. A. Mount) (1 fl. oz./acre = 29.7 ml/min; 1 psi = 6.894757 kPa).
[2] Mean value calculated as aerosol cloud drift velocity (1 mph = 1.609 km/h).
[3] Mean value calculated after Haugen et al. (1961) (1 ft. = 0.3048 m).

All but 2 of the stability ratios reported by these investigators were positive, thus indicating ground-based inversions with warmer air at the higher elevation.

Schatmeyer and Urone (1973) studied the influence of wind velocity, stability ratio, downwind distance, and caged mosquito elevation on kill of adult female *Ae. taeniorhynchus,* and a summary of their results is presented in Table 4. Of 6 evenings, each consisting of 3–4 runs, the highest mean mosquito kill (75%) obtained at 3 ft. elevation was during a strong ground-based inversion. This inversion occurred during evening 5, which produced the highest mean stability ratio of any evening. Mean mosquito kill at 3 ft. of elevation for the other evenings ranged from 25 to 62% even though stability ratios were negative for evenings 2 and 6. Runs during evening 5 also produced only 3% mosquito kill at 50–100 ft. of elevation compared with 84–89% kills at these elevations during evenings 4 and 6. Thus, these results showed that the strong inversion during evening 5 retarded vertical movement of the aerosol cloud beyond 30 ft. of elevation. A low level of turbulence also restricted aerosol elevation during evening 5. Measures of turbulence for evenings 1–4 and 6 were about equal but were much greater than those for evening 5 (Schatmeyer and Urone 1973). In contrast to evening 5, mosquito kills at 50–100 ft. of elevation were about equal to kills at 3 ft. of elevation during evenings 4 and 6 at 300 ft. downwind (Table 4). Schatmeyer and Urone concluded from their model that mosquito kill was inversely related to the vertical atmospheric turbulence and spreading effects produced by vertical differences in horizontal wind velocity.

SWATH

The effective swath of ULV ground aerosol applications is determined by droplet size, insecticide rate, meteorology, and target environment. Most aerosols are applied in urban and suburban areas where a network of streets allows coverage of a target area large enough to provide several days of mosquito control before retreatment is required because of mosquito reinfestation. In small target areas of less than 1 mi.^{2}, application may have to be made more frequently to provide satisfactory control. With most applications, insecticide flow rates are usually set to provide an effective swath of 1 or 2 city blocks. Thus, most of the studies reviewed herein included observations of caged mosquitoes or counts of natural mosquito populations over swaths of 300–600 ft. However, several investigators have used swaths >600 ft. against mosquitoes in California pastures.

Droplet size: Results by Mount et al. (1968, 1970b) and Haile et al. (1982) shown in Table 1 show that the effective swath of an aerosol application is related to droplet size. Against caged adult female *Ae. taeniorhynchus,* a VMD range of 8–15 μm provided an effective swath (92% mean kill) of 300 ft. at a dose of 8.5–12 fl. oz./mi. of 95% malathion, whereas a VMD range of 16–24 μm was effective (92% mean kill) for only 150 ft.

Insecticide rate: Numerous investigators have demonstrated the effect of an insecticide rate on the effective swath. Mount et al. (1968, 1970b) showed that a dose of 4.3 fl. oz./mi. of 95% malathion did not produce an effective swath at any downwind distance, whereas 17 fl. oz./mi. was effective for 600 ft. even when the VMD was above the optimum range. Mount et al. (1968, 1970b) also reported 85–100% mean kill or reduction for 300-ft. swaths with 6–12 fl. oz./mi. of 85% naled against caged and natural populations of *Ae. taeniorhynchus* in an open field and citrus grove, respectively, but no effective swath with only 3 fl. oz./mi. of 85% naled.

Stains et al. (1969) demonstrated the effect of an increased insecticide dose by dispersing massive

rates of 396 and 446 fl. oz./mi. of 85% naled and 6 lb. AI/gal chlorpyrifos, respectively, to achieve effective swaths of 1–2 mi. against caged adult *Culex tarsalis* Coquillett at Skaggs Island, Sonoma County, California, with flat, open terrain and 6–8-mph winds. Also at Skaggs Island, Husted et al. (1975) obtained 87% mean kill of caged *Cx. pipiens* at 150–1,000 ft. downwind with 8–10 fl. oz./mi. of 6-lb. AI/gal chlorpyrifos.

Against caged adult *Culex quinquefasciatus* Say in Arkansas, Thompson and Meisch (1977) showed that 6–12 fl. oz./mi. of 2-lb. AI/gal permethrin provided effective swaths (93–100% kill) of 300 ft., whereas 4 fl. oz./mi. was effective for only 100 ft. In another Arkansas study, Sandosky et al. (1983) reported data indicating that 10.8 fl. oz./mi. of 1.5-lb. AI/gal resmethrin plus 4.5 lb. AI/gal piperonyl butoxide had an effective swath (90–99.9% kill of caged *An. quadrimaculatus*) of 300 ft., whereas 5.4 fl. oz./mi. did not produce 90% kill at any distance.

Womeldorf and Mount (1977) obtained effective swaths of 1,320 ft. with 60 and 118 fl. oz./mi. of 5% pyrethrins plus 25% piperonyl butoxide and 25% resmethrin, respectively, against natural populations of *Aedes nigromaculis* (Ludlow) in California pastures. Also, rates of 24–36 fl. oz./mi. of 26% bendiocarb produced 800–1,125-ft. swaths in California pastures (Miller et al. 1982, Townzen et al. 1987).

Against caged *Ae. taeniorhynchus* exposed in a moderate to heavily vegetated Florida citrus grove, Curtis and Mason (1988) showed that 21.6 fl. oz./mi. of 85% naled provided an effective swath (88–100% kill) of 500 ft., whereas 7.2 fl. oz./mi. was effective (94% mean kill) for only 100 ft. These results suggest that an insecticide rate higher than the label rate is required for 90% or more adult mosquito control in moderate to heavily vegetated target areas.

Meteorology: The results by Schatmeyer and Urone (1973) shown in Table 4 indicated that a strong ground-based inversion (evening no. 5) characterized by stable air and reduced turbulence greatly enhanced mosquito kill over downwind distances of 100–500 ft. Although Curtis and Mason (1988) associated downwind distance with mosquito kill, they were unable to show a correlation between wind velocity and mosquito kill. Some of the variation in mosquito kill in their tests may have been caused by other meteorological factors, such as low-level atmospheric stability and turbulence, that were not measured.

Vegetation and other obstacles: Moderate to dense vegetation and other obstacles, such as homes and solid walls or fences, will limit the effective swath of an aerosol application. Mount et al. (1968) used 12 fl. oz./mi. of 85% naled to reduce a natural population of *Ae. taeniorhynchus* >90% in moderately dense citrus groves over a 300-ft. swath, whereas only 6 fl. oz./mi. was needed to kill >90% of caged adult female *Ae. taeniorhynchus* exposed in an open field. Taylor and Schoof (1971) also obtained twice the level of kill of 3 species of mosquitoes exposed to 95% malathion aerosols over 600-ft. swaths in an open area as those exposed in a moderately dense wooded area. Caged *Psorophora columbiae* (Dyar and Knab) mosquitoes exposed to 93% fenitrothion aerosols in the center of a wide privet hedge were killed at only half the rate of those exposed in the open (Walker et al. 1981). Curtis and Mason (1988) obtained >90% kill of caged adult female *Ae. taeniorhynchus* over 500-ft. swaths in a moderately to densely vegetated citrus grove with 21.6 fl. oz./mi. of 85% naled, whereas the labeled rate of 7.2 fl. oz./mi. provided only 34–58% kill. Rathburn and Dukes (1989) observed 2.5 times more droplets with >3 times greater volume when 91% malathion aerosols were sampled in an open residential area compared with a densely vegetated residential area. Floore et al. (1991) showed that aerosols of technical malathion and 18% resmethrin plus 54% piperonyl butoxide were more efficacious against caged adult *Ae. taeniorhynchus* and *Cx. quinquefasciatus* exposed in an open residential area than in a moderately vegetated residential area. Also, Linley and Jordan (1992) obtained higher percentage kills of caged *Cx. quinquefasciatus* exposed to aerosols of malathion, naled, and resmethrin plus piperonyl butoxide in open than in vegetated terrain.

Droplet collection studies by Rathburn and Dukes (1989) and Brown et al. (1993a) indicated that, although droplet density was greatly reduced by vegetation, the droplet size of malathion aerosols was only slightly smaller when collected in vegetation than in the open. Thus, the application of insecticidal aerosols with a smaller droplet size than 8–15 μm VMD would not likely reduce the limiting effect of vegetation on swath and overall mosquito kill.

Dense housing can also limit the swath of ULV aerosols. In Thailand, Pant et al. (1971) used swaths of only ≈150 ft. for indoor application to kill adult *Aedes aegypti* (Linn.) They obtained 1-day, 3-day, and 5-day posttreatment reductions of 82–99%, 74–89%, and 56–63%, respectively, in numbers of adult female mosquitoes collected on humans with aerosol applications of 95% malathion. A portion of the malathion aerosol was blown indoors by moving a vehicle-mounted ULV generator as close as possible past the open doors and windows of the houses and commercial buildings in target villages. The aerosol generator was moved at a speed of ≈3 mph with the nozzle discharge to the side facing the open windows and doors. Flow rates of technical malathion were 3.5–4.4 fl. oz./min, which produced a dose of 70–80 fl. oz./mi., a rate near that required for effective ULV aerial sprays of malathion against *Ae. aegypti* in Thailand (Lofgren et al. 1970a, 1970b) and >4 times greater than the rate required for 90% kill of caged adult mosquitoes exposed in open terrain.

SPEED

When an effective insecticide dose (fl. oz./mi.) and appropriate atomization are maintained for a designated swath, dispersal speed is not a factor affecting efficacy. Results by Mount et al. (1970b) indicated no difference in the effectiveness of 95% malathion aerosols dispersed at 10 and 20 mph with equivalent doses. Moreover, many investigators, including Mount and Pierce (1971, 1972b, 1976), Mount et al. (1974c, 1975a, 1975b), Rathburn and Boike (1975), Fultz and Carter (1980), and Dukes et al. (1990), have used dispersal speeds of 2.5–20 mph to vary the dose of various insecticides being evaluated for efficacy against adult mosquitoes. Factors that determine the appropriate speed for ground aerosol application are driving conditions in the target area, atomization capacity of the aerosol generator, and insecticide label specifications. The primary reason for higher speeds is, of course, greater coverage capability with each application unit. With speed-correlated insecticide metering systems on ULV aerosol generators, vehicle speed can be varied within calibrated limits (for example, 5–20 mph) and still maintain a constant insecticide dose without any adjustment by the operator. However, with automated operation, droplet size will vary somewhat with change in flow rate as discussed previously. Thus, the droplet sizes of maximum and minimum flow rates within a designated speed range must be determined to ensure atomization within the optimum range and compliance with insecticide labels. Devices for automatic adjustment of nozzle air pressure or rotational speed to maintain constant droplet size output with variation in vehicle speed and insecticide flow rate are technically feasible, but their use on aerosol generators has not been reported.

ASSAY METHODS

The principal method of evaluating the efficacy of insecticidal aerosols has been with caged adult female mosquitoes. There are several advantages in using caged mosquitoes to determine the efficacy of ground aerosols instead of using natural, free-flying mosquito populations. The caged mosquito method provides rapid, economical, and standardized evaluation, whereas assays of natural populations of mosquitoes require additional resources. Also, results with natural population assays can be less certain because of mosquito reinfestation following aerosol application, especially in small target areas of less than 1 mi.^{2}

Comparison of results with caged and free-flying mosquitoes: Results from direct comparisons of caged mosquito and free-flying population methods of assay justify the use of caged mosquitoes. Against *Ae. taeniorhynchus* in Florida, Mount et al. (1966) obtained similar kills of caged wild female adult mosquitoes (70–76%) and reductions in free-flying natural populations (62–75%) simultaneously exposed to HV aerosols (both thermal and nonthermal) of 15 fl. oz./mi. of 85% naled in densely vegetated citrus groves. Also, Pant et al. (1971) obtained 91% kill of caged adult *Cx. quinquefasciatus* (cited as *Culex fatigans*) placed inside houses and exposed simultaneously to the malathion aerosols that reduced the natural population of *Ae. aegypti* by 90%. Against *Ps. columbiae* (cited as *Psorophora confinnis* Lynch Arribalzaga) in Lonoke, AR, a town of ≈1.6 mi.^{2} of typical residential area with moderately dense vegetation, Mount et al. (1972) dispersed 18 fl. oz./mi. of 95% malathion and obtained similar results with caged wild mosquitoes (96% kill) and free-flying natural populations of mosquitoes (91–94% reduction) exposed simultaneously to the aerosol applications. Also, in California pastures, Womeldorf and Mount (1977) observed kills (58–100%) of caged *Cx. quinquefasciatus* that were similar to reductions (69–96%) of natural *Ae. nigromaculis* populations from simultaneous exposure to aerosols of synergized pyrethrins and resmethrin. Finally, against *Ae. taeniorhynchus* in a 50-acre residential beach community in Crescent Beach, FL, Mount et al. (1978b) showed that kills (59–70%) of caged adult females and 45-min posttreatment reductions (56–72%) of a natural population were essentially the same from simultaneous exposure to propoxur aerosols applied at 57–114 fl. oz./mi. of a 1-lb. AI/gal formulation. With aerosol applications of 7.2 fl. oz./mi. of 85% naled in the same community, the percentage kill of caged mosquitoes was somewhat less than the percentage reduction of the natural population (65 versus 85%).

Cage materials: Various cage materials have been tested for insecticide droplet penetration and mosquito kill efficiency. Mount et al. (1966) described a double compartment cage separated by a plastic slide mechanism that was used successfully for many years to evaluate ULV ground aerosols of insecticide. One side of the cage consisted of a 1.75-in. × 5.5-in. cylindrical plastic tube lined with clean paper and covered with a plastic screen on 1 end, whereas the opposite side consisted of an equal size cylindrical tube of 16-mesh galvanized screen wire. During aerosol exposure, all mosquitoes were confined to the screen portion of the cage with the slide in the closed position and the screen end of the plastic tube covered with masking tape to prevent contamination. After aerosol exposure, the tape was removed, the slide was opened to blow mosquitoes into the uncontaminated plastic tube for posttreatment kill observations, and then the slide was closed. Breeland (1970) used a variety of metal, plastic, and nylon screens in ULV aerial spray droplet penetration tests and observed that nylon and galvanized screen were almost equal to unscreened controls. In another ULV aerial spray study, Mount et al. (1970c) observed higher kill (91%) of mosquitoes exposed in 16-mesh galva-

nized screen wire cages than kill (6–37%) in 32- or 60-mesh galvanized screen wire cages or kill (6–66%) in various fabric cages including nylon. Townzen and Natvig (1973) used 18-mesh nylon net for construction of disposable cages routinely used in field assays with adult mosquitoes. Rathburn et al. (1989) obtained essentially the same percentage kill of adult female *Ae. taeniorhynchus* and *Cx. quinquefasciatus* exposed to ULV ground aerosols in the Townzen and Natvig disposable cages of cardboard and 18-mesh nylon net and their standard metal cages with 14- × 18-mesh bronze screen wire. However, Rathburn et al. used CO_2 anesthesia to transfer all mosquitoes to clean holding cages for observation following aerosol exposure, whereas Townzen and Natvig left mosquitoes in the exposure cages for observation of kill. Boobar et al. (1988) reported that aerosol droplet penetration of sentinel cages was directly related to the percentage of open area in the screen materials. With ULV aerosols of fenitrothion and bendiocarb, Bunner et al. (1989a) showed that kill of adult female *Ae. aegypti* was slightly less (68–75%) when mosquitoes were transferred to clean cages following exposure than when left in the contaminated exposure cages (72–87%).

Cage configuration, orientation, and placement: In addition to cage materials, the cage design, orientation to the prevailing wind, and placement height can influence mosquito assay results. Rathburn et al. (1969) demonstrated the effect of cage orientation and placement on results with ULV aerial sprays of insecticide. With a flat cage design, they obtained higher kills with vertical (34 and 87%) than horizontal (16 and 23%) cages at both ground level and 6 ft. above the ground, respectively. Rathburn et al. also indicated no difference in results with flat and cylindrical cages. With applications of ULV ground aerosols of synergized pyrethrins and resmethrin against caged *Cx. quinquefasciatus* in California pastures, Womeldorf and Mount (1977) obtained higher kill of mosquitoes in cages placed at 3 ft. above the ground (92%) than at 0.5 ft. above the ground (73%). Similarly, Tapley et al. (1980) obtained 91 and 77% kill of caged mosquitoes of 8 different species at heights of 5.3 and 1.3 ft., respectively, that were exposed to aerosols of 25% bendiocarb. On the basis of wind tunnel tests, Bunner et al. (1989b) suggested that a cylinder, screened on all sides, with the longitudinal axis perpendicular to the ground would provide a consistent cage profile to the wind, regardless of wind direction.

INSECTICIDE EFFICACY

The efficacy of potential mosquito adulticides is determined initially in laboratory wind-tunnel tests. New insecticides are compared against a standard adulticide, usually malathion. Those insecticides with a toxicity equal to or greater than the standard are considered for field trials. Other factors for consideration prior to field testing include mammalian toxicity, potential nontarget effects, and commercial availability.

Laboratory wind-tunnel tests: A summary of the relative toxicities of nonthermal aerosols of mosquito adulticides tested in laboratory wind tunnels is presented in Table 5. Because of the variation in methods, materials, and measures of toxicity in various reports, results are given as the reciprocal ratio of each insecticide to malathion and are listed in order of decreasing toxicity. The most toxic mosquito adulticides were synergized pyrethrins and the pyrethroids, deltamethrin, permethrin, fluvalinate, resmethrin, and phenothrin. Note that the toxicities of permethrin and resmethrin were increased by ≈4-fold when synergized with piperonyl butoxide. Against *Ae. taeniorhynchus,* Mount et al. (1974a) showed that the maximum toxicity of pyrethrins was achieved with a 1:5 ratio of insecticide to piperonyl butoxide, whereas the toxicity of resmethrin was enhanced with each ratio increase from 1:1 to 1:25 of resmethrin to piperonyl butoxide. With pyrethrins and the pyrethroids, toxicities varied considerably among genera with *Anopheles* spp. having the highest reciprocal ratios to malathion. The 2 carbamates, bendiocarb and propoxur, were intermediate in toxicity, and the 5 organophosphates, fenitrothion, fenthion, chlorpyrifos, naled, and malathion, were the least toxic. Reciprocal ratios to malathion among genera did not vary more than 2.6-fold with any of the carbamate or organophosphate adulticides.

Effective rates in field tests: In all insecticide efficacy field studies including 3 or more discriminating doses, probit analysis (Raymond 1985) was used to estimate rates of insecticide needed for 90% mosquito control. With 2 doses, probit paper was used to estimate the 90% effective rate. Thus, effective doses for 90% control indicated in this summary may differ slightly from those shown in some of the original reports. When only 1 dose was tested that produced 90% or more control, that dose is included in the tables. For convenience and comparability, data from all studies were converted, when necessary, to indicate application speed in mph, flow rate in fl. oz./min, and rate in lb. AI/acre. Although much of the insecticide is not deposited because aerosols are space treatments, the quantity of insecticide per unit area is a convenient and comparable term for indicating the rate needed for satisfactory control. Insecticide flow rates in units of time or distance are also commonly used to indicate the insecticide rate; however, flow rates must be associated with a swath to be meaningful. Effective rates are based on insecticide concentration, flow rate, vehicle speed, and kill of caged or reduction of natural populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath, unless otherwise indicated in the tables.

Results of successful tests: The rates of ULV

Table 5. Summary of relative toxicities of nonthermal aerosols of insecticides to adult mosquitoes in laboratory wind-tunnel tests (after Mount et al. 1970a, 1971, 1974b; Mount and Pierce 1973, 1975; Pierce et al. 1973; Coombes et al. 1977; Zboray and Mount 1977; Rathburn et al. 1982; Magnuson et al. 1985; Floore et al. 1992).

Insecticide	Reciprocal ratio to malathion				
	Aedes spp.[1]	*Anopheles* spp.[2]	*Culex* spp.[3]	*Psorophora* sp.[4]	Mean
Deltamethrin	77.5	—	—	18.0	47.8
Permethrin + PBO, 1:5	—	42.8	—	—	42.8
Resmethrin + PBO, 1:5	13.5	52.0	18.5	7.4	22.9
Permethrin	9.4	13.0	—	9.7	10.7
Pyrethrins + PBO, 1:5	9.4	15.5	5.2	5.8	9.0
Fluvalinate	0.6	—	16.7	—	8.7
Resmethrin	3.2	16.5	3.7	—	7.8
Phenothrin	1.2	—	14.2	—	7.7
Bendiocarb	4.7	—	3.1	—	3.9
Propoxur	2.9	5.0	—	3.2	3.7
Fenitrothion	2.9	—	4.6	2.6	3.4
Fenthion	4.7	2.9	1.8	2.6	3.0
Chlorpyrifos	3.1	2.8	2.9	2.0	2.7
Naled	2.2	2.1	2.4	1.8	2.1
Malathion[5]	1.0	1.0	1.0	1.0	1.0

[1] *Aedes aegypti* (Linn.), *Ae. nigromaculis* (Ludlow), and *Ae. taeniorhynchus* (Wied.).
[2] *Anopheles albimanus* Wied. and *An. quadrimaculatus* Say.
[3] *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say.
[4] *Psorophora columbiae* (Dyar and Knab).
[5] In tests using the same methods and materials, concentrations of malathion were 428, 364, 388, and 140 ppm for 90% kill of *Aedes, Anopheles, Culex,* and *Psorophora* spp., respectively, in 12–24 h posttreatment.

ground aerosols of insecticide observed to provide 90% or more kill of caged mosquitoes or, in a few cases, reduction of natural mosquito populations are presented in Tables 6–10 and are summarized in Table 11. With 3 exceptions, Table 11 summarizes all of the results presented in Tables 6–10. These exceptions were with malathion and included the indoor applications by Pant et al. (1971) and Perich et al. (1990) and the morning tests with marginal meteorological conditions reported by Mount and Pierce (1976). Also, 3 exceptions to 90% or more control (72–85%) are footnoted in Tables 6–9. The flow rates in Tables 6–11 reflect only the undiluted insecticide formulations as indicated and do not include any diluents although some investigators used diluents in their tests, especially with pyrethroid insecticides.

Insecticides summarized in Table 11 are listed in order of decreasing efficacy. Results show that 5 pyrethroids, including cyfluthrin, deltamethrin, lambda cyhalothrin, cypermethrin, and synergized phenothrin, were the most effective insecticides tested as ULV ground aerosols against adult mosquitoes. Other highly effective pyrethroids were fenvalerate and fluvalinate. Highly efficacious pyrethroids that have US-EPA registration include synergized phenothrin, synergized permethrin, synergized pyrethrins, permethrin, synergized resmethrin, phenothrin, and resmethrin. One highly effective carbamate, bendiocarb, is US-EPA registered for use. Organophosphate adulticides requiring higher doses than the pyrethroids include fenthion, chlorpyrifos, naled, and malathion. These insecticides have been registered by US-EPA for >25 years as ULV ground aerosols to control adult mosquitoes.

With most of the adulticides, the US-EPA maximum label rate equals or exceeds the effective rate shown for each mosquito genus in Table 11. The maximum label rate for synergized pyrethrins and unsynergized or synergized permethrin, phenothrin, and resmethrin is 0.007 lb. AI/acre, which equals or exceeds the effective rate for each of these adulticides tested against *Anopheles* and *Culex* spp. However, against *Aedes* spp., unsynergized phenothrin and resmethrin had effective rates above the maximum label rate of 0.007 lb. AI/acre. The effective rate of 0.0079 lb. AI/acre for synergized resmethrin against *Aedes* spp. was only slightly more than the label rate, whereas no data for synergized phenothrin against *Aedes* spp. have been reported. With bendiocarb, the effective rate (0.006 lb. AI/acre) for *Aedes, Anopheles,* and *Culex* spp. was only ≈one-half the maximum label rate of 0.011 lb. AI/acre. The maximum label rate of 0.03 lb. AI/acre for fenthion exceeds the effective rates for *Aedes, Anopheles,* and *Culex* spp. by almost 3-fold, which allows the application of rates that may be effective even when meteorological conditions are marginal or when aerosols are applied in moderately dense vegetation. The maximum label rate of 0.02 lb. AI/acre for naled is essentially the same as the effective rate for each genus. With malathion, effective rates for each genus were less than the maximum label rate of 0.054 lb. AI/acre.

Results of unsuccessful tests: Not all trials with ground ULV aerosols of insecticide have been highly successful in controlling adult mosquitoes.

Table 6. Rates of ultralow-volume (ULV) ground aerosols of 91–96% malathion (Cythion®, Fyfanon®) for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
Aedes taeniorhynchus (Wied.)	Florida	Open field	Curtis 55,000[4]	5	0.63	0.061 c	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	6.02	0.076 c	Mount et al. (1970b)
Culex quinquefasciatus Say	Texas	Open field	Leco ULV	10	1.58	0.020 c	McNeill and Ludwig (1970)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	2.00	0.025 c	Mount and Pierce (1971)
Aedes aegypti (Linn.)	Thailand	Indoors	Leco ULV	3	4.00	0.326 n[5]	Pant et al. (1971)
Ae. taeniorhynchus	Georgia	Residential	Leco ULV	10	3.77	0.048 c	Taylor and Schoof (1971)
Anopheles albimanus (Wied.)	Georgia	Residential	Leco ULV	10	2.37	0.030 c	Taylor and Schoof (1971)
Cx. quinquefasciatus	Georgia	Residential	Leco ULV	10	4.69	0.059 c	Taylor and Schoof (1971)
Ae. taeniorhynchus	Florida	Residential	Leco ULV	5	1.35	0.034 c	Rathburn and Boike (1972a)
Culex nigripalpus Theobald	Florida	Residential	Leco ULV	5	3.01	0.076 c	Rathburn and Boike (1972a)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	5.63	0.071 c	Mount and Pierce (1972b)
Psorophora columbiae (Dyar and Knab)	Arkansas	Residential	Leco HD-ULV	10	3.00	0.038 c,n	Mount et al. (1972)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.76	0.060 c	Mount et al. (1974c)
Ae. taeniorhynchus, Aedes sollicitans (Walker)	N. Carolina	Residential	Leco HD-ULV	10	4.30	0.054 n	Axtell and Dukes (1974)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.93	0.062 c	Mount et al. (1975b)
Anopheles quadrimaculatus Say	Florida	Open field	Leco HD-ULV	10	4.22	0.053 c	Mount et al. (1975b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.07	0.051 c	Mount et al. (1975c)
Ae. taeniorhynchus	Florida	Open field	Micro-Gen MS	10	4.65	0.059 c	Mount et al. (1975c)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.40	0.030 c	Mount et al. (1975c)
An. quadrimaculatus	Florida	Open field	Micro-Gen MS	10	3.80	0.048 c	Mount et al. (1975c)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	12.85	0.162 c	Mount and Pierce (1976)
Ae. taeniorhynchus	Florida	Open field	Lond. Aire XW	10	15.15	0.191 c	Mount and Pierce (1976)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	9.30	0.117 c	Mount and Pierce (1976)
An. quadrimaculatus	Florida	Open field	Lond. Aire XW	10	9.83	0.124 c	Mount and Pierce (1976)
Ae. taeniorhynchus	Florida	Residential	Various[6]	10	2.13	0.027 c	Rathburn and Boike (1977)
Cx. nigripalpus	Florida	Residential	Various[6]	10	2.13	0.027 c	Rathburn and Boike (1977)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.40	0.017 c	Rathburn and Boike (1981)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Rathburn and Boike (1981)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.00	0.024 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.00	0.024 c	Roberts (1982)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	3.00	0.036 c	Walker and Meisch (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.24	0.015 c	Roberts (1983)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.84	0.010 c	Roberts (1983)
Ae. aegypti	Dom. Rep.	Indoors	Leco HD-ULV	5	9.00	0.218 c[7]	Perich et al. (1990)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Floore et al. (1991)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Floore et al. (1991)
Ae. aegypti	Malaysia	Residential	Leco HD-ULV	5	3.01	0.076 c[8]	Vythilingam and Panart (1991)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Modified for ULV application.
[5] Based on a 150-ft. swath.
[6] Includes Leco® HD-ULV, Micro-Gen® LS2-15, London Aire® XW, Buffalo Turbine® Sonic, Micro Mist®, and Tifa® 100-E-ULV.
[7] Mosquito kills of 100 and 44% outside and inside of houses, respectively.
[8] Mosquito kills of 92 and 39% outside and inside of houses, respectively.

Table 7. Rates of ultralow-volume (ULV) ground aerosols of chlorpyrifos, fenitrothion, fenthion, and naled for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Chlorpyrifos, 6 lb. AI/gal (Dursban®, Mosquitomist®)				
Culex quinquefasciatus	Texas	Open field	Leco ULV	10	1.25	0.005 c	McNeill and Ludwig (1970)
Aedes taeniorhynchus	Florida	Open field	Leco ULV	10	1.81	0.014 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.56	0.020 c	Mount and Pierce (1972b)
Psorophora columbiae	Arkansas	Residential	Leco HD-ULV	15	3.20	0.017 n	Mount et al. (1972)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.05	0.008 c	Rathburn and Boike (1975)
Culex nigripalpus	Florida	Residential	Leco HD-ULV	10	1.66	0.013 c	Rathburn and Boike (1975)
Cx. quinquefasciatus	California	Open field	Military[4]	10	1.33	0.010 c	Husted et al. (1975)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.34	0.010 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	2.13	0.017 c	Rathburn et al. (1981)
Cx. quinquefasciatus	California	Open field	Custom built	6	2.00	0.008 c[5]	Townzen et al. (1987)
			Chlorpyrifos, 12%				
Aedes aegypti	Mexico	Open field	Leco 500	10	6.50	0.008 c	Velazquez-Quintana et al. (1995)
Cx. quinquefasciatus	Mexico	Open field	Leco 500	10	6.50	0.008 c	Velazquez-Quintana et al. (1995)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	1.49	0.019 c	Mount and Pierce (1971)
Anopheles quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	2.22	0.029 c	Walker et al. (1981)
Ps. columbiae	Arkansas	Open field	Leco HD-ULV	10	2.22	0.029 c	Walker et al. (1981)
			Fenitrothion, 95% (Sumithion®, Accothion®)				
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	2.13	0.028 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	2.13	0.028 c	Rathburn et al. (1981)
			Fenthion, 93% (Baytex®)				
Ae. taeniorhynchus	Florida	Open field	Leco ULV	15	0.95	0.008 c	Mount et al. (1970b)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	0.97	0.012 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.07	0.013 c	Rathburn and Boike (1975)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.07	0.013 c	Rathburn and Boike (1975)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.45	0.018 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.79	0.010 c	Mount et al. (1987a)
			Naled, 85%, 14 lb. AI/gal (Dibrom®)				
Ae. taeniorhynchus	Florida	Open field	Curtis 55,000[4]	5	0.39	0.014 c	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Wooded	Curtix 55,000[4]	5	0.65	0.024 n	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	15	1.01	0.012 c	Mount et al. (1970b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.37	0.025 c	Mount and Pierce (1972b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.37	0.025 c	Mount and Pierce (1972b)
Ps. columbiae	Arkansas	Residential	Leco HD-ULV	10	1.20	0.020 n	Mount et al. (1972)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.81	0.033 n	Mount and Pierce (1974)

Table 7. Continued.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Naled, 85%, 14 lb. AI/gal (Dibrom®)				
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	5	0.60	0.020 n[6]	Mount et al. (1978b)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	0.75	0.012 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	0.75	0.012 c	Rathburn et al. (1981)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Modified for ULV application.
[5] Based on 1,000-ft. swath.
[6] Forty-five-minute posttreatment reduction of 85%.

Possible reasons for mediocre results or control failure include 1) inadequate insecticide dose, 2) mosquito resistance to the insecticide used, 3) unfavorable meteorological conditions, 4) inadequate coverage of the target area because of dense vegetation and other obstacles or an incomplete road network, and 5) rapid mosquito reinfestation of the target area.

Turner (1977) reported that ULV ground aerosols of 96% malathion had limited practicability for control of *Anopheles farauti* Laveran, the principal vector of malaria on the island of Guadalcanal in the Solomon Islands. He applied 0.036 lb. AI/acre (17 fl. oz./mi.) to 102 villages at 10-day intervals with a Leco HD-ULV aerosol generator and obtained a mean reduction in man-biting collections of 72% at 1-day posttreatment compared with the mean 1–3-day pretreatment collections. Collections returned to pretreatment levels at 2–6 days posttreatment, which indicated rapid reinfestation of the villages. Unfavorable weather, including heavy rains, strong winds, and wrong wind direction, during some of the aerosol applications also contributed to the inadequate levels of mosquito control.

Strickman (1979) was only moderately successful in reducing oviposition rates of *Cx. pipiens* and *Cx. restuans* at 2 target sites of ≈0.14 and 0.23 mi.^{2} in Decatur, IL, with 3 ground aerosol applications of 52.7 fl. oz./mi. of 91% malathion at 2–3-wk intervals. Strickman's results indicated mean reductions of 52, 47, and 31% in numbers of egg rafts deposited in pails of water treated with alfalfa pellets on 0, 1, and 2 nights posttreatment. Possible reasons for mediocre results include small treatment sites, long intervals between aerosol applications, a high rate of mosquito reinfestation, and tolerance of *Culex* spp. to malathion.

Fox (1980), Fox and Specht (1988), and Chadee (1985) observed the lack of effectiveness of ULV ground aerosols of 4.3 fl. oz./min of 95–96% malathion dispersed from Leco HD-ULV aerosol generators against populations of *Ae. aegypti* in San Juan, Puerto Rico, and St. Joseph, Trinidad, West Indies. At a dispersal speed of 10 mph and swath of 300 ft. (not stated), the application rate would have been 0.054 lb. AI/acre. Possible reasons for ineffectiveness include malathion resistance (Fox and Bayona 1972), inadequate insecticide rate, and mosquito protection from aerosols by dense vegetation, solid fencing, and housing. A rate of 0.326 lb. AI/acre was used by Pant et al. (1971) for successful control of *Ae. aegypti* in Thailand, a rate 6-fold greater than the 0.054 lb. AI/acre rate used in Puerto Rico and Trinidad. Also, in Trinidad, applications were begun at 5 p.m. when, no doubt, unstable air and turbulence would diffuse much of the aerosol upward and above mosquito habitat.

Parsons (1982) reported that weekly applications of 7.2–12 fl. oz./mi. of 85% naled were ineffective in controlling natural populations of mosquitoes (species not indicated) in Fort Meade, FL. Actually,

Table 8. Rates of ultralow-volume (ULV) ground aerosols of bendiocarb and propoxur for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Bendiocarb, 25%, 1.67 lb. AI/gal (Ficam®)				
Aedes taeniorhynchus	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Aedes sollicitans (Walker)	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Aedes aegypti	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Anopheles albimanus	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Culex pipiens	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Culex quinquefasciatus	Louisiana	Open field	Micro-Gen ED	10	3.60	0.008 c	Tapley et al. (1980)
Aedes nigromaculis (Ludlow)	California	Pasture	Leco HD-ULV	5	2.50	0.003 n[4]	Miller et al. (1982)
Anopheles freeborni Aitken	California	Pasture	Leco HD-ULV	5	2.50	0.003 c[4]	Miller et al. (1982)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	2.00	0.003 c[4]	Miller et al. (1982)
Culex tarsalis Coquillett	California	Pasture	Leco HD-ULV	5	2.00	0.003 c[4]	Miller et al. (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.62	0.010 c	Roberts (1984)
Anopheles quadrimaculatus	Florida	Open field	Leco HD-ULV	10	3.85	0.008 c	Roberts (1984)
Ae. nigromaculis	California	Pasture	Micro-Gen[5]	5	4.00	0.005 n[4]	Townzen et al. (1987)
Cx. quinquefasciatus	California	Pasture	Micro-Gen[5]	5	4.00	0.010 c[4]	Townzen et al. (1987)
			Propoxur, 1–2 lb. AI/gal (Baygon®)				
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	12.96	0.034 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	18.88	0.025 c	Mount et al. (1975b)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	12.78	0.017 c	Mount et al. (1975b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.01	0.009 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.91	0.006 c	Mount et al. (1978a)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	5	9.50	0.024 n[6]	Mount et al. (1978b)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	9.12	0.024 c	Walker et al. (1981)
Psorophora columbiae	Arkansas	Open field	Leco HD-ULV	5	5.79	0.018 c	Walker et al. (1981)
An. quadrimaculatus	Arkansas	Wooded	Leco HD-ULV	5	7.00	0.021 c	Walker et al. (1981)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	8.52	0.011 c	Rathburn et al. (1981)
Culex nigropalpus	Florida	Residential	Leco HD-ULV	10	8.52	0.011 c	Rathburn et al. (1981)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Based on 300–2,200-ft. swaths.
[5] Model not specified.
[6] Forty-five-minute reduction of 72%.

Table 9. Rates of ultralow-volume (ULV) ground aerosols of pyrethrins and resmethrin for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
Pyrethrins, 4–12% plus piperonyl butoxide, 12–60% (Pyrenone®, Synerol®)							
Aedes taeniorhynchus	Florida	Open field	Leco HD-ULV	10	8.89	0.0086 c	Mount and Pierce (1972b)
Ae. taeniorhynchus, Aedes sollicitans	NC	Residential	Leco HD-ULV	10	4.00	0.0019	Axtell and Dukes (1974)
Anopheles albimanus	El Salvador	Residential	Leco HD-ULV	5	2.50	0.0024 n	Hobbs (1976)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	5.60	0.0027 c	Rathburn and Boike (1975)
Culex nigripalpus	Florida	Residential	Leco HD-ULV	10	3.30	0.0016 c	Rathburn and Boike (1975)
Aedes nigromaculis	California	Pasture	Leco HD-ULV	5	10.24	0.0057 n[4,5]	Womeldorf and Mount (1977)
Culex quinquefasciatus	California	Pasture	Leco HD-ULV	5	5.51–10.24	0.0057 c[4]	Womeldorf and Mount (1977)
Anopheles quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	3.00	0.0016 c	Walker and Meisch (1982)
Culex tarsalis	California	Pasture	Custom built	10	15.70	0.0015 c[4]	Townzen et al. (1988)
Resmethrin, 2–3.34 lb. AI/gal (SBP-1382®)							
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	10.30	0.0268 c	Mount and Pierce (1971)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	5	0.84	0.0073 c	Rathburn and Boike (1972b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	16.32	0.0710 c	Mount and Pierce (1972b)
Ae. nigromaculis	California	Pasture	Leco HD-ULV	5	9.83	0.0058 n[4]	Womeldorf and Mount (1977)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	9.83	0.0058 c[4]	Womeldorf and Mount (1977)
Aedes vexans (Meigen)	Minnesota	Wooded	Custom built	10	1.61	0.0070 n	Sjogren and Frank (1979)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.38	0.0036 c	Rathburn et al. (1981)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	0.80	0.0070 c	Sandoski et al. (1983)
Psorophora columbiae	Arkansas	Open field	Leco HD-ULV	10	0.80	0.0070 c	Stark et al. (1985)
Resmethrin, 0.9–1.5 lb. AI/gal plus piperonyl butoxide, 4.5 lb. AI/gal (Scourge®, Oblique®)							
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	8.56	0.0100 c	Mount et al. (1974c)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.57	0.0030 c	Mount et al. (1974c)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	8.89	0.0024 c[4]	Womeldorf and Mount (1977)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	0.90	0.0035 c	Sandoski et al. (1983)
Ps. columbiae	Arkansas	Open field	Leco HD-ULV	10	0.92	0.0018 c	Stark et al. (1985)
Aedes melaniman	California	Residential	Custom built	5	2.00	0.078 c	Townzen et al. (1987)
Cx. quinquefasciatus	California	Pasture	Custom built	5	4.00	0.0035 c[4]	Townzen et al. (1987)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	3.00	0.0059 c	Floore et al. (1991)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	3.00	0.0059 c	Floore et al. (1991)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	1.35	0.0020 c	Weathersbee et al. (1991)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	0.68	0.0010 c	Groves et al. (1994)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Dose based on 660–2,640-ft. swaths.
[5] Landing rate reductions were 75–76%.

Table 10. Rates of ultralow-volume (ULV) ground aerosols of pyrethroid insecticides for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Bioresmethrin, 15% plus piperonyl butoxide, 15%				
Aedes taeniorhynchus	G. Cayman	Swamp	Mod. Tifa	10	3.20	0.0027 n[4]	Brooke et al. (1974)
			Cyfluthrin, 3.2 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.02	0.0001 c	Roberts (1985)
Anopheles quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.04	0.0002 c	Roberts (1985)
			Cypermethrin, 2.48 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.44	0.0014 c	Roberts (1983)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.06	0.0002 c	Roberts (1983)
			Deltamethrin, 2%, 0.167 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.26	0.0005 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.09	0.00002 c	Roberts (1982)
			Fenvalerate, 2.4 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.36	0.0042 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.34	0.0010 c	Roberts (1982)
			Fluvalinate, 2 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.50	0.0117 c	Kline et al. (1986)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.38	0.0010 c	Kline et al. (1986)
			Lambda cyhalothrin, 2%				
Culex tarsalis	California	Open desert	Micro-Gen G-9HD	5	1.13	0.0003 c[4]	Schaefer et al. (1990)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	1.55	0.0003 c	Weathersbee et al. (1991)
			Permethrin, 2–5 lb. AI/gal (Punt®)				
Culex quinquefasciatus	Arkansas	Open field	Leco HD-ULV	10	1.21	0.0030 c	Thompson and Meisch (1977)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.91	0.0076 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.36	0.0009 c	Mount et al. (1978a)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	0.50	0.0013 c	Walker and Meisch (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.79	0.0037 c	Kline et al. (1986)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.21	0.0010 c	Kline et al. (1986)
Cx. quinquefasciatus	California	Pasture	Custom built	7	4.25	0.0045 c[4]	Townzen et al. (1987)
Cx. tarsalis	California	Pasture	Custom built	7	4.25	0.0045 c[4]	Townzen et al. (1987)
			Permethrin, 12–31% plus piperonyl butoxide, 20–66% (Permanone®, Biomist®, Flak®)				
Aedes sollicitans	New Jersey	Open field	Beecomist HD	5	0.50	0.0033 c	McNelly and Benzon (1995)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	15	0.80	0.0007 c	Groves et al. (1994)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	15	0.80	0.0017 c	Groves et al. (1994)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	10	0.53	0.0035 c	Meisch et al. (1997)

Table 10. Continued.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
		Phenothrin, 40%, 3.04 lb. AI/gal (Sumithrin®, Solo® 40-OS)					
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.74	0.0206 c	Roberts (1981)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.36	0.0014 c	Roberts (1981)
		Phenothrin, 20% (Sumithrin®, Solo® 40-OS) plus piperonyl butoxide, 50%					
An. quadrimaculatus	Arkansas	Open field	VecTec Grizzly	15	0.93	0.0012 c	Groves et al. (1994)
Psorophora columbiae	Arkansas	Open field	VecTec Grizzly	15	0.93	0.0012 c	Groves et al. (1994)

[1] Ten miles per hour is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Dose based on 600–>2,640-ft. swath.

Parsons showed that a mean reduction in CDC light trap and truck trap collections of 50% was achieved during the nights of treatment. However, essentially the same levels of reduction were consistently observed at 2 untreated sites. Because the untreated sites were only ≈2,000–3,000 ft. from the large target treatment area of ≈3 mi.^{2}, these untreated sites may have also been treated with the naled aerosol. Nevertheless, the results of Parson's tests suggest that a higher rate of naled may be required for highly effective mosquito control in Fort Meade and comparable communities.

In New Orleans, LA, Focks et al. (1987) observed 73 and 75% reduction in mean adult captures and oviposition rates, respectively, of *Ae. aegypti* populations subjected to 11 sequential aerosol applications of 24 fl. oz./mi. of 91% malathion at 12-h intervals over a 5.5-day period. The authors suggested that overcrowded housing and dense vegetation hampered aerosol drift and effectiveness. Another possible reason for mediocre results could have been unfavorable meteorological conditions because aerosol applications were made during the morning (6:00–7:15 a.m.) when conditions are usually calm and late afternoon (5:30–6:45 p.m.) when unstable air would diffuse much of the aerosol above mosquito habitat. Furthermore, the 0.048 lb. AI/acre rate of malathion used in this study would have to be increased 2- or 3-fold to provide a high degree of mosquito kill in dense vegetation.

Against caged wild *An. quadrimaculatus* in Arkansas, Weathersbee et al. (1989) obtained 75% control with 0.0012 lb. AI/acre of synergized resmethrin (Scourge®, Clarke Engineering Technologies Inc.) and only 49% control with 0.012 lb. AI/acre of fenthion. However, subsequent results with synergized resmethrin showed adequate control (90% or more) of *An. quadrimaculatus* in Arkansas at rates of 0.001–0.002 lb. AI/acre (Weathersbee et al. 1991, Groves et al. 1994). Poor results with fenthion are likely a result of mosquito resistance to this insecticide because previous tests with fenthion against caged mosquitoes from a laboratory colony indicated satisfactory results at 0.01 lb. AI/acre (Mount et al. 1978a).

Efird et al. (1991) reported only 42% kill of caged wild *An. quadrimaculatus* in Arkansas with a rate of 0.05 lb. AI/acre of malathion. They indicated the possibility of resistance of this species to malathion as suggested by unpublished results of laboratory topical application studies. Also, in the same field studies, they obtained only 77% mosquito kill with 0.0015 lb. AI/acre of synergized permethrin, whereas Groves et al. (1994) showed ≈90% kill of caged wild *An. quadrimaculatus* in Arkansas with synergized permethrin at 0.0007–0.0017 lb. AI/acre (Table 10). Thus, meteorological conditions may have been less favorable in the 1991 tests than in the 1994 tests.

Groves et al. (1997) reported 45–75% control of caged wild mosquitoes of 3 different species and

Table 11. Summary of rates for ultralow-volume (ULV) ground aerosols of undiluted insecticide for 90% or more control of adult mosquitoes in open to moderately open terrain with favorable meteorological conditions.

Insecticide[1]	Lb. AI/gal	% AI	Lb. AI/acre (fl. oz./min at 10 mph for 300-ft. swath)[2]				
			Aedes spp.	*Anopheles* spp.	*Culex* spp.	*Psorophora* spp.	Mean
Cyfluthrin	3.2	—	0.0001 (0.02)	0.0002 (0.04)	—	—	0.00015 (0.03)
Lambda cyhalothrin	—	2%	—	0.0003 (1.55)	0.0003 (1.55)	—	0.00030 (1.55)
Deltamethrin	—	2%	0.0005 (2.26)	0.00002 (0.09)	—	—	0.00035 (1.38)
Cypermethrin	2.48	—	0.0014 (0.44)	0.0002 (0.06)	—	—	0.0008 (0.25)
Phenothrin + PBO[3]	1.52 + 4.4	20 + 50%	—	0.0012 (0.93)	—	0.0012 (0.93)	0.0012 (0.93)
Fenvalerate	2.4	—	0.0042 (1.36)	0.0010 (0.34)	—	—	0.0026 (0.84)
Bioresmethrin + PBO	—	15 + 15%	0.0027 (0.32)	—	—	—	0.0027 (0.32)
Permethrin + PBO	2.89 + 6.09	31 + 66%	0.0036 (0.95)	0.0020 (0.53)	—	—	0.0028 (0.74)
Pyrethrins + PBO	—	12 + 60%	0.0047 (3.62)	0.0020 (1.54)	0.0029 (2.23)	—	0.0032 (2.46)
Permethrin	3.60	—	0.0057 (1.22)	0.0011 (0.24)	0.004 (0.85)	—	0.0036 (0.77)
Resmethrin + PBO	1.5 + 4.5	18 + 54%	0.0079 (4.05)	0.0024 (1.23)	0.0039 (2.00)	0.0018 (0.92)	0.0040 (2.05)
Bendiocarb	1.67	—	0.006 (2.73)	0.006 (2.73)	0.006 (2.73)	—	0.0060 (2.73)
Fluvalinate	2.0	—	0.0117 (4.50)	0.0010 (0.38)	—	—	0.0064 (2.46)
Phenothrin	3.04	40%	0.021 (5.20)	0.0014 (0.35)	—	—	0.011 (2.75)
Fenthion	9.67	93%	0.013 (1.03)	0.010 (0.79)	0.013 (1.03)	—	0.012 (0.95)
Resmethrin	3.34	40%	0.028 (6.44)	0.0070 (1.61)	0.0056 (1.29)	0.007 (1.61)	0.012 (2.76)
Chlorpyrifos	6.00	—	0.012 (1.54)	—	0.010 (1.28)	0.017 (2.18)	0.013 (1.67)
Propoxur	1.00	—	0.021 (16.16)	0.017 (13.08)	0.011 (8.46)	0.018 (13.85)	0.017 (13.08)
Naled	14.00	85%	0.020 (1.20)	—	0.016 (0.96)	0.020 (1.20)	0.019 (1.14)
Fenitrothion	9.67	93%	0.024 (1.90)	0.029 (2.30)	0.028 (2.22)	0.029 (2.36)	0.028 (2.22)
Malathion	9.70	95%	0.042 (3.32)	0.033 (2.62)	0.039 (3.10)	0.038 (3.00)	0.038 (3.00)

[1] Insecticide formulations listed here are maximum or near maximum concentrations currently registered by US-EPA for ULV ground aerosol application. Other ULV formulations of the same insecticide with similar concentrations may also be registered for use and even more concentrated formulations of some insecticides may be available in the future. However, substantially lower concentrations of some insecticides are also registered, but would represent low-volume (LV) or high-volume (HV) application even though labels indicate ULV application. Dilution of the insecticide formulations listed in this table by the applicator would also represent LV or HV applications rather than ULV application. Common names of insecticides are used in this table. See preceding tables for trade names of insecticides.

[2] 1 lb. AI/acre = 1.12 kg AI/ha; 1 fl. oz./min = 29.7 ml/min; 1 mph = 1.609 km/h; 1 ft. = 0.3048 m.

[3] PBO = piperonyl butoxide.

genera with rates of 0.00175 lb. AI/acre of synergized permethrin (Permanone®, Fairfield American Corp., Frenchtown, NJ) and resmethrin (Scourge) and 0.001 lb. AI/acre of synergized prallethrin (Responde®). The likely explanation for these unsatisfactory results is insufficient insecticide rate. The 0.00175 rate for both synergized permethrin and resmethrin is substantially less than effective rates (averages of 0.0025 and 0.0047 lb. AI/acre, respectively) reported previously for these insecticides (see Tables 9–11).

NONTARGET EFFECTS

One factor that limits the potential nontarget effects of ULV ground aerosols is that effective insecticide rates for adult mosquitoes are relatively low compared with rates used for other types of insect control. A 2nd factor is that aerosols consisting of small droplets are space treatments, and only a small portion of the insecticide is deposited on the ground or vegetation in the target area. Tucker et al. (1987) reported that only 1.4–2.0% of fenthion and 16–17% of malathion aerosols were deposited on filter papers placed 25 ft. downwind at ground level. Also, Moore et al. (1993) showed that only 1–17% of malathion aerosols was deposited on filter papers placed at 50–300 ft. downwind at ground level. The potential nontarget effects of ULV ground aerosols of insecticide that have been studied include those on human targets, beneficial animals, automotive paint, and noise level of aerosol generators.

Human targets: Moore et al. (1993) demonstrated that quantities of ULV malathion aerosols deposited on humans were inconsequential. Stationary and moving human subjects were exposed to a rate of 4.3 fl. oz./min of 91% malathion dispersed at 10 mph at downwind distances of 5–50 ft. Their results indicated that estimated levels of malathion dermal exposure were less than the acute lethal dose for human subjects by 4 orders of magnitude or more.

Nontarget animals: In tests with mammals and birds, Joseph et al. (1972) observed no mortality or red cell cholinesterase inhibition of caged bobwhite quail, *Colinus virginianus,* white laboratory mice, and containerized goldfish exposed to 18 and 180 (10 times the labeled rate) fl. oz./mi. of 95% malathion aerosols. Also, Mallack et al. (1975) demonstrated that 95% malathion aerosols rates that were 5–20 times the labeled rate (130–520 fl. oz./mi.) did not inhibit brain acetycholinesterase levels in mature chickens and New Zealand rabbits.

Several studies on fish, shrimp, crabs, and other marine organisms have been reported. Tagatz et al. (1974) reported no kill of confined blue crabs, *Callinectes sapidus*; grass shrimps, *Palaemonetes vulgaris* and *Palaemonetes pugio*; pink shrimp, *Penaeus duorarum*; or sheepshead minnows, *Cyprinodon variegatus,* exposed to 26 fl. oz/mi. of 95% malathion aerosols. Furthermore, brain acetylcholinesterase activity was not reduced in sheepshead minnows exposed to the aerosols. In a series of studies with ULV ground aerosol applications of fenthion at 6 fl. oz./mi., Clark et al. (1985) reported mean kills of 79 and 83% of caged *Ae. taeniorhynchus* and *Cx. quinquefasciatus* mosquitoes at downwind distances of 50–300 ft. Deaths of grass shrimp, *P. pugio,* and pink shrimp were attributed to low salinity and dissolved oxygen rather than to exposure to fenthion aerosols (Borthwick and Stanley 1985). Also, Tagatz and Plaia (1985) observed no difference in average numbers of individuals and species, primarily annelids and arthropods, in estuarine benthic communities located in fenthion-treated and untreated sites. Moore et al. (1985) demonstrated that fenthion from the aerosol applications did not accumulate to a detectable level (0.010 μg/g) in tissues of caged shrimp or fish. In tests with caged mysids, *Mysidopsis bahia,* McKenney et al. (1985) obtained mean kills of 32 and 50% in control and exposed sites, respectively, following 3 fenthion aerosol applications on different dates. They suggested that the insecticide reduced growth of the mysids by 24% on the basis of dry weight measurements 180 days posttreatment. Furthermore, Tucker et al. (1987) observed no kill of common snook juveniles, *Centropomus undecimalis*; tarpon snook juveniles, *Centropomus pectinatus*; sheepshead minnow juveniles, *Cyprinodon variegatus,* or adult calanoid copepods, *Acartia tonsa,* exposed to aerosol applications of 24 fl. oz./mi. of 91% malathion, 6 fl. oz./mi. of 93% fenthion, or 8 fl. oz./mi. of 85% naled.

In studies on nontarget insects, Washino et al. (1977) used pretreatment and 24-h posttreatment deZulueta 24-ft.^{2} net collections to measure 31–81% reduction in 3 different test sites and a 78% increase in a 4th test site of natural populations of Cicadellidae exposed to ULV aerosols of 0.006 lb. AI/acre of synergized pyrethrins or 0.003–0.006 lb. AI/acre of synergized resmethrin. With aerosol applications against caged honeybees, Caron (1979) reported 39–68% kill at 50–100 ft. with 95% malathion, 14–33% kill at 50–200 ft. with 10% naled, and essentially no kill at any exposure distance with 5% pyrethrins. However, he observed that routine night applications of these insecticides had no discernible effect on honeybee colonies.

Automotive paint: Rathburn and Boike (1972a) reported no visible damage to paint on panels furnished by General Motors Corporation that were exposed at only 10 ft. from the line of travel of an aerosol generator dispersing 26 fl. oz./mi. of 95% malathion. Also, Mount et al. (1978a) observed no visible damage to automotive paints exposed to aerosols of 6 fl. oz./mi. of 2 lb. AI/gal of permethrin or 54 fl. oz./mi. of 1.67 lb. AI/gal propoxur. From results of a laboratory study, Tietze et al. (1992) indicated a positive correlation between malathion droplet size and paint damage spot size. Laboratory

settling chamber tests revealed that size thresholds of malathion droplets too small to cause visible damage averaged 8 and 11 μm diameter on washed 1K (basecoat: 872-AB921; clearcoat: RK7103) and 2K (basecoat: 872-AB921; clearcoat: RK7100) black paint standards supplied by E. I. DuPont De Nemours and Company (Troy, MI). In field tests, they observed no visible damage to 1K and 2K paints, but microscopic damage was found on paint panels placed on the hood, trunk, and doors of an automobile when parked parallel or perpendicular to the course of the aerosol generator and when driven through the aerosol cloud from a stationary generator.

Aerosol generator noise: Most ULV aerosol generators produce considerable noise and some may exceed the United States Occupational Safety and Health Administration (US-OSHA) 8-h hearing hazard criteria of 90 dBA when engines are operated at a high RPM. Nelson et al. (1975) measured the noise levels at the operator's ear from several generators mounted on a ¾-ton truck. Noise levels were 87–105 dBA for 2 generators equipped with vortex nozzles (Micro-Gen MS2–15 and Leco HD-ULV), 83–105 dBA for a generator with a pneumatic nozzle (Buffalo Turbine Sonic), and 72 dBA for a generator equipped with a rotary nozzle (Beecomist Systems Cardinal 150 ULV). In another study, noise produced by Leco HD-ULV and Micro-Gen ED2–20A aerosol generators was measured at 70–78 dBA when the generators passed by a microphone at 10 mph (Morton 1980). Robinson and Ruff (1991a, 1991b) and Robinson (1994) reported that 3 generators with vortex nozzles and rotary air compressors (London Fog 18–20 ULV, Curtis Dyna-Fog Maxi-Pro 4 ULV, and VecTec Grizzly ULV) produced ≥91 dBA when operated at high engine RPM (≥2,200 for London Fog, ≥1,800 for Curtis Dyna-Fog, and ≥1,950 for VecTec). A generator with a pneumatic nozzle and piston air compressor (Conner Engineering Bison ULV) was shown to be relatively quiet with only 83 dBA at 3,000 engine RPM, the highest level tested (Robinson and Ruff 1992). However, the quietest unit was a rotary nozzle generator (Beecomist Systems Pro-Mist 25HD ULV), with only 63 dBA at a maximum nozzle RPM of 33,500 (Robinson et al. 1993).

CONCLUSIONS

1. ULV ground aerosol applications of insecticide are as efficacious against adult mosquitoes as HV or LV aerosols. The degree of adult mosquito kill obtained with any insecticide application is related to the dose of active ingredient and many other application and environmental factors but not to application volume. Inert ingredients such as water or oil diluents do not kill mosquitoes and only add cost and inconvenience to ground aerosol operations. Dilution of an insecticide formulation would be required only if the flow rate of the undiluted formulation is substantially less than 0.5 fl. oz./min. Some insecticide labels that require dilution should be modified to allow ULV application of the undiluted formulation. ULV technology offers an increased insecticide payload for more rapid application and increased safety by elimination of dense fogs created by HV thermal atomization.

2. ULV aerosols with an optimum droplet size spectrum can be produced by several types of nozzles including vortex, pneumatic, and rotary. Droplet size is dependent primarily on nozzle air pressure or nozzle rotation speed and secondarily on insecticide flow rate.

3. Label flow rates of insecticide for ULV aerosol application can be delivered accurately (within ≈6%) during routine operations with speed-correlated metering systems within a calibrated speed range, usually not exceeding 20 mph.

4. The most economical and convenient method of droplet size determination for ULV aerosols of insecticide is the waved-slide technique. This simple technique uses Teflon-covered glass microscope slides, a micrometer disc in an ocular objective on a compound microscope for measurement of size, and calculation of VMD based on droplet diameter because droplet impingement is a function of diameter. Other techniques that have been used successfully for droplet size determination are settlement chamber, cascade impactor, Coulter Counter, hot wire, and laser.

5. The efficacy of ULV ground aerosols against adult mosquitoes is related to droplet size because it governs air transport and impingement. The optimum droplet size for mosquito adulticiding is 8–15 μm VMD on the basis of laboratory wind-tunnel tests and field research with caged mosquitoes.

6. In general, ULV aerosols should be applied following sunset when mosquitoes are active and meteorological conditions are favorable for achieving maximum levels of mosquito control. However, with favorable or even marginal meteorological conditions, application can be made successfully during daytime hours when nighttime application is impractical. During marginal meteorological conditions, application rates may have to be increased to achieve satisfactory results. The critical meteorological factors are wind velocity and direction, temperature, and atmospheric stabililty and turbulence. Wind velocities of 1–7 mph, with gusts not exceeding 11 mph, are the most suitable for aerosol drift across target swaths. A ground-based inversion with a low level of turbulence will optimize aerosol cloud diffusion across the target swath. However, successful mosquito kill can be achieved even during slightly unstable conditions if a prevailing wind exists.

7. Maximum effective swaths are obtained with aerosols in the optimum VMD range during favorable meteorology in open to moderately open terrain. In general, the insecticide rate must be in-

creased in direct proportion to an increase in swath to maintain the same level of mosquito control.

8. Dispersal speed within a range of 2.5–20 mph is not a factor affecting efficacy if insecticide rate and optimum atomization are maintained.

9. Percentage kills with caged mosquito assays are comparable with reductions in free-flying natural populations. For the most consistent results with the caged method, mosquitoes should be exposed in 14–18-mesh cylindrical cages, screened on all sides, with the longitudinal axis perpendicular to the ground and at a height 2–6 ft. above the ground.

10. The field efficacies of mosquito adulticides applied as ULV ground aerosols are predictable from the results of laboratory wind-tunnel tests.

11. Results of field tests in open to moderately open terrain during favorable meteorological conditions indicated that ULV insecticidal aerosol application rates producing 90% or more control of *Anopheles, Culex,* and *Psorophora* spp. are below or ≈equal to maximum US-EPA label rates. Against some *Aedes* spp., some pyrethroid insecticides must be synergized to be 90% or more effective at label rates.

12. Results of field tests in residential areas with moderate to dense vegetation and in citrus groves or other densely wooded areas showed that insecticide rates of ULV ground aerosols must be increased 2–3-fold to obtain 90% or more control of adult mosquitoes. However, the maximum rates on some insecticide labels would have to be increased to allow higher application rates.

13. Applications of ULV ground aerosols of insecticide in accordance with label directions following sunset do not pose a serious threat to humans, nontarget beneficial animals, or automotive paints.

14. Some aerosol generators operated at high RPM levels exceed the US-OSHA 8-h hearing hazard criteria of 90 dBA and may require hearing protectors for operators.

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FAILURE OF AN INSECT ELECTROCUTING DEVICE TO REDUCE MOSQUITO BITING[1]

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ABSTRACT. Insect electrocuting devices using ultraviolet light as an attractant were tested for their ability to reduce mosquito biting in backyards. Biting collections were made in 6 adjacent backyards located in South Bend, Indiana. Two of the yards were equipped with electronic insect killing devices, 2 with CDC traps, and 2 had no apparatus. The collections were made on 8 nights from June through August 1982. The insects killed by the electrocuting devices also were collected. Only 3.3% of the 3212 insects killed on an average night were female mosquitoes. Humans in the vicinity of the electrocuting devices were consistently more attractive to mosquitoes than the devices. Even after 11 days of continuous operation, the electrocuting devices failed to reduce the mosquito biting rate.

INTRODUCTION

Electronic insect killing devices using an electrocuting grid and ultraviolet (UV) light as an attractant (commonly sold as Zappers,®, Bugwackers,®, Bug Blasters,® etc.) frequently are used in an attempt to control pest insects in backyards, campgrounds, swimming pools and other outdoor recreation areas. These devices are advertised on the basis of claims by the manufacturers that they have a "lure range" of a certain distance depending on the power of the UV source, they can clear insects from a certain radius around the trap, and they disrupt the "breeding cycle" of insects. Test data available from some of the manufacturers indicate that, if they are the sole source of light, these devices are useful in reducing indoor house fly populations. Unfortunately, no data are presented on the efficiency of these traps outdoors or their ability to reduce mosquito biting; the purpose for which most of the traps are purchased. This study was designed to evaluate the ability of these devices to reduce mosquito biting in backyard situations.

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MATERIALS AND METHODS

The study was conducted from late June through August 1982 in a suburban neighborhood on the northeast side of South Bend (St. Joseph County), Indiana. The area was known to have moderate to heavy local populations of *Aedes vexans* (Meigen) and *Ae. trivittatus* (Coquillett) (St. Joseph County Mosquito Abatement Program, Unpublished data). The testing was conducted in 6 backyards within the neighborhood. The backyards were adjacent to each other in a straight north to south line. Each backyard was approximately 35 m square. The 6 yards were surrounded on the north, south, and east by similar residential areas. Adjacent to the yards to the west was a small, shallow drainage ditch (ca. 1 m wide) frequently containing water and intermittently producing *Ae. trivittatus*. Immediately to the west of the drainage ditch was a 5 to 15 m wide woodlot running almost the entire length of the backyards. The woodlot consisted of large, mixed deciduous trees (trunk diam. 10–40 cm) and a dense herbaceous understory. To the west and south of the woodlot was a schoolyard consisting of mowed grass. Immediately to the north of the woodlot was an unmowed field with vegetation ranging from 0.5 to 2 m high. The land-

scaping in the individual yards varied from completely open mowed grass to yards containing ornamental shrubs, ground cover and small vegetable gardens, to wooded yards with large deciduous trees (trunk diam. 30–60 cm) and a relatively complete canopy.

The electrocuting device chosen for the study was the Sears model 833.1432 Electronic Insect Killer.[2] This device consists of a 25 watt UV bulb surrounded by an electrocuting grid inside a protective screen and rain shield, and is similar to the design of most electronic insect killers. It is advertised as having a "lure range of 279 m^2 (3200 ft^2, ca. ¾ acre) and as being effective over a radius of 30.5 m (100') from the trap. The operating instructions recommend that the devices be placed "25–50 ft away from the area of human activity and nearby insect infestation to draw insects away."

In the first series of tests, yards were chosen at random and 2 yards were equipped with the electrocuting devices, 2 yards were equipped with standard 6 volt CDC traps, and 2 yards contained no device. The electrocuting devices were suspended 2.5 m above the ground in the center of the yards and were turned on 36 hr prior to the collection of biting mosquitoes. Mosquitoes coming to human bait were collected in each yard from 1 hr before to 1 hr after sunset on 6 nights of mild weather (wind 0–8 km/h. RH 75–95%, temp. 15–24°C). The 2 hr experimental period was divided into six 15 min collecting periods with 5 min between periods. The individuals serving as bait were stationed 8 m east of the electrocuting devices and CDC traps (on the opposite side of devices from the wooded area) and in a similar position in the yards with no apparatus. The collectors captured mosquitoes coming to bite during the 15 min periods with a mouth aspirator. After each 15 min period, the collectors moved to different yards to adjust for individual variation in attractiveness to mosquitoes and collection efficiency. The possible effects of landscaping and trap placement in the yards were controlled by moving the traps to different yards during each night of testing. As a result, each yard was tested with the electrocuting device, CDC trap or no apparatus twice during the experiments.

The biting rate in each yard was determined by totalling the number of mosquitoes collected coming to bite during the six 15 min periods and expressing that number as a percent of the total collected in the yard with the maximum number for the night. Expressing the biting rate as a percent of the maximum allows comparison of results between nights despite variations in the absolute number collected due to climatic and other factors.

The number of insects killed by an electrocuting device was determined by collecting the insects in a fine mesh nylon net bag attached to the bottom of the device. Collections of this type were made during the 2 hr test periods on 5 of the 6 nights of testing. In addition, the insects killed by the electrocuting devices were collected during 5 daylight periods (0700–1900 h) and five 24 hr periods to determine what proportion of the insects were killed during the daylight hours, how many and what kind of insects other than mosquitoes were killed, and the proportion of mosquitoes to non-mosquitoes killed.

A second series of tests was performed to determine if long-term use of the electrocuting devices (as recommended by the manufacturer) reduced mosquito biting. An electrocuting device was suspended in the center of 2 of the yards (chosen at random) and run 24 hr daily for 10 and 11 days prior to conducting 2 nights of human bait collections in the manner described above. Statistical analysis of data used the methods described in Sokal and Rohlf (1969).

RESULTS

The electrocuting devices killed an average of 3212 insects per 24 hr period during the 5 collections that were made (Table 1). Of these, the vast majority (89%) were small non-mosquito nematocerous Diptera. Several other types of insects were found in the collections, including mosquitoes which comprised 6.4% of the total kill. Approximately half of the mosquitoes killed were female and constituted only 3.3% of the total insects killed.

An average of 66 insects were killed during each of the 6 daylight periods sampled (Table 1). Non-mosquito Nematocera were the most frequently killed insects (36%) followed closely by muscoid flies (33%). An average of less than 2 mosquitoes were killed per period, constituting 2.7% of the daytime kill.

During the 2 hr samples taken during the twilight periods when the biting collections were made, an average of 2163 insects were killed by the electrocuting devices (Table 1). Non-mosquito Nematocera were the most frequently killed insects (88%). Mosquitoes comprised 4.3% of the total insects killed with females making up slightly more than half of this number. CDC traps, because of their low pow-

[2] Sears Roebuck and Company, Chicago, IL 60684. Mention of trade names is for identification only and does not constitute endorsement by NIH or the University of Notre Dame.

Table 1. Average number and percentage of the total number of insects killed by electrocuting devices during 24 hr periods (n=5), daylight periods from 0700 hr to 1900 hr (n=6), and the 2 hr test periods (n=10).

	Average number of insects killed		
	24 hr period	Daylight period	2 hr test period
Coleoptera	68.8 (2.1)*	6.3 (8.1)	79.0 (3.7)
Lepidoptera	37.2 (1.2)	0.6 (0.9)	1.5 (<.1)
Hemiptera	9.2 (0.3)	1.2 (1.8)	20.4 (0.9)
Homoptera	2.4 (<.1)	1.2 (1.8)	0.9 (<.1)
Ephemeroptera	—	—	0.2 (<.1)
Trichoptera	41.2 (1.3)	1.0 (1.5)	41.0 (1.9)
Hymenoptera	1.8 (<.1)	3.2 (4.9)	2.4 (0.1)
Muscoid flies	43.2 (4.5)	21.3 (32.5)	9.6 (0.4)
Empididae	11.8 (0.4)	0.3 (0.5)	11.2 (0.5)
Non-mosquito Nematocera	2863.2 (89.1)	23.7 (36.2)	1905.9 (88.1)
♀ mosquitoes	106.8 (3.3)	1.0 (1.5)	49.0 (2.3)
♂ mosquitoes	100.6 (3.1)	0.8 (1.2)	42.3 (2.0)
Total insects	3211.8	65.6	2163.4

* Percent of total number of insects killed.

ered light source, collected very few insects during the twilight test periods.

During the first series of tests, in which the electrocuting devices were turned on 36 hr prior to making the biting collections, 1143 mosquitoes were collected coming to bite in the yards equipped with the electrocuting devices, 1096 in the yards with the CDC traps, and 1110 in the yards with no apparatus. There was no difference in the biting rate experienced in the yards with the electrocuting devices, with CDC traps, and with no apparatus (ANOVA $P > .05$, Table 2). The biting rates in the yards with electrocuting devices ranged from 31% to 100% of the maximum. The yards with CDC traps ranged from 44% to 100% of the maximum, and the yards with no apparatus ranged from 40% to 100% of the maximum.

In the yards equipped with the electrocuting devices, 1089 female mosquitoes were collected from the human bait and 489 were killed by the electrocuting devices (Table 3). The majority of those collected coming to bite were *Ae. trivittatus* while *Ae. vexans* was the species most frequently killed in the electrocuting devices.

When the number of mosquitoes coming to bite in the yards equipped with the electrocuting devices is compared to the number killed in the electrocuting devices on an individual basis, the number coming to bite exceeded the number killed by the electrocuting devices in every case (Table 4).

The second series of tests, in which the electrocuting devices were run continuously for 10–11 days prior to making biting collections, produced results similar to those of the previ-

Table 2. Mosquito biting rate experienced in yards equipped with electrocuting devices, CDC traps, and no apparatus. The rate in each yard is a percent of the number collected in the yard with the highest total for the night. The traps were moved to different yards for each trial and were turned on 36 hr prior to testing in test 1. In test 2, the traps were left in the same yards and run 10-11 days prior to testing.

	Percent of maximum biting rates						
Trial	Electrocuting device		CDC trap		No apparatus		Maximum #@
Test 1*	1	2	1	2	1	2	
1	66	57	100	75	75	84	44
2	57	31	50	77	100	74	68
3	87	97	58	100	40	94	62
4	89	100	62	82	87	55	219
5	85	100	88	86	91	63	172
6	61	58	100	44	83	87	154
Test 2**							
1	68	86	100	79	51	49	85
2	46	100	38	66	47	31	55

* No difference in biting rate between areas with electrocuting devices, CDC traps, and no apparatus (ANOVA $P>.05$).

** No difference in biting rate between areas (Kruskal-Wallis Ranks Test $P>.05$).

@ Maximum is the highest number of mosquitoes collected coming to bite in a yard during the test period.

Table 3. Number of mosquitoes collected coming to bite in a backyard with an electrocuting device and the number killed in the device. Total of ten 2 hr test periods.

Species	Number killed or collected	
	Electrocuting device	Human bite
Ae. vexans ♀	174	211
Ae. vexans ♂	126	—
Ae. trivittatus ♀	11	738
Ae. trivittatus ♂	—	—
Other species ♀*	304	140
Other species ♂*	297	—
Total mosquitoes	489	1089

* Including unidentified specimens.

ous tests. A total of 211 mosquitoes was collected coming to bite in the yards with the electrocuting devices, 209 in the yards with the CDC traps, and 128 in the yards with no apparatus. The biting rate did not differ in yards with electrocuting devices, CDC traps, or no apparatus (Kruskal-Wallis Ranks Test $P > .05$, Table 2).

Marked differences were detected, however, in the number of mosquitoes collected by the individuals serving as bait. Of the 10 persons volunteering their services throughout the study, the average collection rates ranged from a low of 0.4 mosquitoes/min to 1.29 mosquitoes/min. Although only marginally significant (ANOVA $P = .09$, Kruskal-Wallis Test $P \leq .05$) there was a noticeable trend in the biting rates experienced in the different yards, regardless of the presence or absence of electrocuting devices. Though somewhat variable, the biting rate in certain yards was consistently higher than the rate detected in other yards, even though they were adjacent to each other.

DISCUSSION

Although many insects were killed by the electrocuting devices used in this study, the devices did not reduce the number of mosquitoes coming to bite. This was true even after the recommended long term use of the traps. These results are consistent with previous evaluations of other similar devices (Surgeoner and Helson 1977; G. B. Craig, Jr., personal communication).

Several factors are responsible for the failure of these devices to reduce mosquito biting. The primary factor is that humans are more attractive to mosquitoes than UV light. This is evident in that, given the same amount of time, the human bait in yards equipped with electrocuting devices collected 1089 female mosquitoes while the electrocuting devices killed only 489 (Table 3). Related to the fact that the electrocuting devices kill few mosquitoes, is the problem that an attempt to control populations of organisms possessing the reproductive potential and dispersal capacity of mosquitoes by killing a few hundred a night, even on a very local basis, is a futile effort at best.

Another major shortcoming of these devices is that not all insects, including mosquitoes, are equally attracted to light. Insects active during the daytime or early evening periods will not be influenced by the presence of the UV light. *Aedes trivittatus* was such a species in this study, rarely occurring in the electrocuting device collections from the evenings and never from the daylight samples. In addition, the total number of insects killed in the daylight samples was only 2% of the number killed during the total 24 hr period.

In summary, the electrocuting devices were not successful in reducing mosquito biting and most of the insects killed were innocuous to humans. It seems probable that mosquito biting in backyards could be influenced more by using personal repellents, changing the type of clothing or soaps used by persons in the area, or by altering the landscaping of the area to remove mosquito resting habitats (shrubbery, heavy ground cover, etc.) from the immediate vicinity, than by the use of UV light attractant electrocuting devices.

Table 4. Number of female mosquitoes collected coming to bite in a yard with an electrocuting device, and the number killed in the device during 5 individual 2 hr tests.

Trial	Number of female mosquitoes captured			
	Site 1		Site 2	
	Electrocuting device	Human bait	Electrocuting device	Human bait
1	31	94	49	90
2	44	146	151	172
3	129	194	30	219
4	15	54	12	60
5	11	39	17	21
Total	230	527	259	562

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VECTOR-COMPETENCE STUDIES FOR BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASE VIRUSES WITH *CULICOIDES VENUSTUS* (CERATOPOGONIDAE)

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ABSTRACT. Field-collected females of *Culicoides venustus* from New York state were tested for oral susceptibility to bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. The infection rates obtained for females exposed to a virus meal were low (BTV 0.7% for 1/141, EHDV 2.6% for 1/38), suggesting that the species would not be an efficient vector of these viruses in New York. Females of *C. venustus* were easy to use in vector competence studies with the same methods used for *C. variipennis;* they were relatively long-lived and readily fed through a membrane and on embryonating chicken eggs. Three other species of *Culicoides* did not take a blood meal under the same conditions except for a few females of *C. stellifer* that were long-lived and assayed negative for infection with BTV.

Several species of *Culicoides* are pests of mammalian livestock in New York State (Schmidtmann et al. 1980). Four of these species, *C. venustus* Hoffman, *C. stellifer* (Coquillett), *C. biguttatus* (Coquillett) and *C. obsoletus* (Meigen), were used in experiments to determine if they could be vectors of bluetongue virus (BTV) or of epizootic hemorrhagic disease virus (EHDV). Of these, only *C. venustus*, a relatively large and robust species, was used successfully in that sufficient numbers of females took blood meals. This species is widespread in the eastern United States with its range extending westward to Wisconsin and southward to Florida (Wirth 1965).

This paper reports preliminary laboratory studies to determine whether *Culicoides* species other than *C. variipennis* (Coquillett), the primary vector of BTV throughout most of the United States (Jones et al. 1981), are potential vectors of BTV. Because the methodology for the use of a species is important, we used several test procedures to determine whether field-collected females of *C. venustus* would be easy to use in arbovirus research.

MATERIALS AND METHODS

Adult *Culicoides* were collected in 1978–79 from a pasture in Tompkins County, New York with light traps baited with CO_2 (dry ice). A serologic survey for antibody to BTV in slaughter cattle had indicated that BT was rare or nonexistent in New York State (Metcalf et al. 1981), and BTV or EHDV have never been reported from the collection area.

Female flies were separated to species, shipped alive lightly chilled, and used in experiments under the same conditions used with *C. variipennis* (Jones and Foster 1978a). They were offered an infective blood meal (1 part cell-culture-adapted virus suspension and 9 parts defibrinated sheep blood) through membranes prepared from the skins of 1-day-old chicks. The blood meal contained about $10^{7.5}$ median cell-culture infectious doses/ml. En-

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ON ASSESSING THE BENEFITS OF PUBLIC MOSQUITO CONTROL PRACTICES[1]

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ABSTRACT. Public mosquito control can be characterized as a nonmarket good whereby the absence of price-quantity information normally used to determine demand and benefits creates a dilemma to the researcher. In response to this dilemma economists have advanced several methods to assess demand components and value of nonmarket goods. The contingent market valuation (CMV) approach is but one method. This paper outlines the relevant theory behind benefit measures, and develops and demonstrates a CMV approach that can be used to value public mosquito control.

INTRODUCTION

Public mosquito control can be characterized as a nonmarket good, whereby it is not sold in a market as are consumer goods (clothing, termite control service, etc.) This absence of market conditions poses a dilemma to economists and public policy makers faced with assessing the value of and concerning decisions about public-nonmarket goods such as public mosquito control. Specifically the lack of numerical data (price-quantity data) normally used to determine demand and the associated value for any particular good creates a nonconventional empirical problem.

In response to this numerical dilemma, economists have advanced several techniques that have been used to assess such nonmarket goods as air and water quality improvements, a variety of recreational activities, health risk-safety, imported fire ant control and gypsy moth control.

The purpose of this paper is to outline the theory and concept of benefits, and demonstrate one approach used to assess the benefits of mosquito control.

METHODOLOGY AND APPROACH

DEMAND AND THE CONCEPT OF BENEFITS. When considering benefits from consuming a good, economists equate the area under the associated demand curve in the absence of income effects with benefits. Points along a demand curve reflect amounts people would be willing to pay rather than forego consumption of the good in question, hence, the area under demand is a representative measure of benefits (Bohm 1976, Mishan 1976, Just et al. 1982). This is easily illustrated by way of Fig. 1.

Demand is represented by the line AB. For the price-quantity combination QoPo gross benefits are approximated by the area OACQo. At that price-quantity combination total costs are represented by the area OPoCQo. The difference between gross benefits and costs measure net benefits, area PoAC and is referred to as consumer surplus, that is, a surplus which accrues to consumers as a net economic benefit. The net benefit represents the net willingness-to-pay (WTP), the difference between what the consumer is willing to pay and the actual amount paid corresponding to the consumer's maximum net WTP. For the one price-quantity combination example, marginal WTP is equivalent to maximum WTP, but with multiple price-quantity combinations, maximum WTP is equal to the sum of marginal WTP.

CONSUMER SURPLUS AS A BENEFIT MEASURE. Although the concept of consumer surplus (CS) may appear straightforward, economists have debated over its appropriateness as a benefit measure for some time (Freeman 1979, Just et al. 1982). The crux of the issue lies with an income effect from price and income changes. A positive income effect will shift the demand curve to the left, represented by AD in Fig. 1. Total and net WTP will tend to overstate gross and net economic benefits in this case. Furthermore, ambiguous measures result depending on the order of price and/or income changes (Just et al. 1982). Recent work has focused on operational techniques to calculate exact welfare measures from demand curves (Hausman 1981, McKenzie 1983, Bergland and Randall 1984).

It must be emphasized that these techniques are based on the existence of demand curves, and hence, assume that price-quantity information are available as in the case of market goods from which demand is estimated. In the case of nonmarket goods, because price-quantity are

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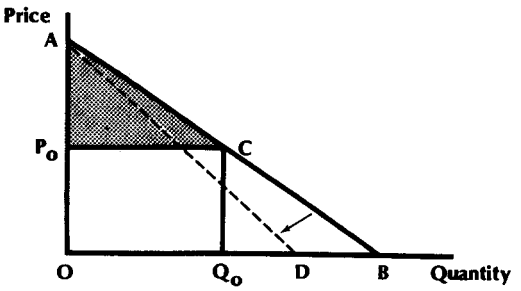

Fig. 1. Demand and consumer surplus.

not observed the researcher must first gather these data using one of the basic nonmarket approaches discussed below.

Although the commonly cited theoretical rationale for the source of discrepancy between exact welfare measures is due to income effects, other reasons have been advanced. Knetsch (1984, 1985), and Knetsch and Sinden (1984) argue that results of empirical studies provide evidence of consistent differences between exact welfare measures for goods that represent a small proportion of total expenditures (Bishop and Heberlein 1979). It is concluded that a discontinuity exists in the value function at the reference point based on experimental evidence (Knetsch 1985).

Some economists have interpreted from the empirical evidence that individuals do not behave as utility maximizers, which underlies demand theory, and hence act irrational (see Opaluch 1984 for a summary). If tastes (descriptions of what an individual likes) and values (description of the way the world ought to be) both influence consumer behavior, that is, enter into the utility function, and if tastes and values are not comparable, the relative influence of values on consumer behavior may offer an explanation of the above divergence and observed preference reversal. In fact, some researchers believe that preferences revealed through a contingent market valuation (CMV) approach, because of the hypothetical nature of the questions, are more influenced from values rather than tastes (Cummings et al. 1986). Recently, Smith and Desvousges (1986) examined disparities among welfare measures and perceived entitlements to risk. They reasoned that the difference in WTP bids to avoid increases in environmental risk and WTP bids to obtain equivalent risk levels from reductions results from traditional utility and welfare maximization whereby this process does not reflect individual's perceived entitlements to various nonmarket goods. Hence, a reformulation of perceived constraints in utility maximization is suggested to accommodate for the disparities in welfare measures.

NONMARKET VALUATION METHODS. In response to the absence of price-quantity data necessary to value nonmarket goods, efforts over the past two decades have resulted in a variety of methods (see Hueth and Strong 1984 for a recent evaluation of nonmarket techniques and Cummings et al. 1986). These techniques can be separated into those that measure WTP indirectly on the basis of actions revealed in the market place (hedonic price, travel cost, and household production function approaches), while the contingent market valuation (CMV) technique measures WTP directly from consumer surveys. The CMV technique is based on the premise of a realistically designed, though hypothetical, market setting. An individual is asked to reveal his/her preference in the form of a bid (maximum amount willing to pay) contingent on the availability of the good in question. Commonly the level of the good is changed in increments and the individual is asked to reveal his corresponding bid in an iterative manner. In a demonstration project, a CMV technique was used to value public mosquito abatement (Ofiara and Allison, unpublished data).

MOSQUITO BENEFIT MEASURE

The approach used in this paper to develop a method to value mosquito control was based on the contingent market valuation (CMV) approach. Because this approach relies on survey data and due to the nature of the CMV approach, questionnaire design forms a most crucial part of CMV applications. The plan of this section will be to present and describe the survey instrument with a primary focus on the benefit valuation questions.

Contingent market valuation allows for flexibility in the good to be valued in the proposed market. However, considerable care must be taken in developing both the good and market so that they appear realistic, credible and are easily understood by the survey respondent. It is especially important to achieve these survey objectives by introducing as little as possible (preferably none) bias or offense to the respondent (Dillman 1978, Desvousges et al. 1983, Rowe and Chestnut 1983).

BACKGROUND DATA. The first part of the questionnaire began with questions about perceptions of mosquitoes as a public health threat and/or nuisance pest; time of day mosquitoes are bothersome; perceptions of mosquitoes relative to other biting insect pests (where respondents ranked these in descending order); amount of leisure time and outdoor activities respondent spends his free time in (e.g., various sports, yard-house work, hob-

bies); outdoor activities that mosquitoes are bothersome to the respondent (ranked in descending order); knowledge about the mosquito control program in their area; private annual expenses on mosquito control/repellent products; socio-economic information; information about expenses for possible medical treatment resulting from mosquito bites; and opinionated questions about mosquitoes and mosquito control.

This sequence of information aided in having the survey respondent think about mosquito control and how mosquitoes affect their lives at the same time establishing a rapport with him/her. This also assisted in creating a favorable atmosphere for the main objective—benefit assessment.

BENEFIT ASSESSMENT. This portion of the questionnaire developed the hypothetical market, defined and described the good to be valued, and the valuation questions. The first part of the introduction to the valuation questions advanced the market setting and the good:

> Mosquito control in your county is provided by a mosquito control agency. This agency is delegated with providing mosquito control for the general public within its jurisdiction, in this case the county. The main objective of mosquito control is to reduce the physical amount (abundance) of mosquitoes. This is accomplished by an appropriate combination of chemical and non-chemical control tools. Benefits that arise from your county control program are related to the public health threat, and nuisance aspect of mosquitos. Many varieties of mosquitoes are carriers of disease, malaria and yellow fever are the most well known diseases. Presently, malaria and yellow fever are no longer a problem in the United States, although the potential for a problem does exist. This is a direct result of mosquito control efforts, and public health efforts. Currently, varieties of the encephalitis complex, a disease, pose threats to public health.
>
> Regarding public health aspects, benefits from control are a reduction in the incidence and mortality of mosquito borne diseases, as well as a reduction in the threat of risk to public health. In regard to the nuisance aspect of mosquitos, benefits from control are fewer mosquitoes landing on you and biting you, resulting in an overall increase in the enjoyment and satisfaction of everyday outdoor activities, as well as recreational activities. In this sense mosquito control has increased the quality of life in areas where mosquitoes are extremely abundant. To provide mosquito control for your county, first a control agency was established, and then materials and equipment were purchased. Money for mosquito control in your county comes from county residents. As a taxpayer, you pay directly for mosquito control through tax dollars each year.

Immediately following this description was information that introduced and described the good, presented along with the key visual aid[4] to help in defining the good:

> Mosquito control agencies have identified various levels of mosquito concentrations in order to assist them in their control decisions, as well as evaluate the performance of their control tools. Here are pictures that show different levels of concentrations of mosquitoes that occur throughout the mosquito season, *and* are obtainable by the mosquito control agency in your county. These pictures were taken during the daytime when most mosquitoes are *not* active feeders. GIVE RESPONDENT PHOTOGRAPHS. A general rule of thumb mosquito agencies use to represent evening or nighttime situations, when mosquitoes are *most* active, is that nighttime situations are *5 to 6 times greater* than daytime situations. That is, nighttime situations are equivalent to multiplying the number of mosquitoes in these photos by 5 or 6. In addition, similar numbers of mosquitoes that appear on the *front* of the volunteer in the pictures, also appear on the *back* of the volunteer, as well as on the rest of the body not shown.
>
> Picture A represents both an outbreak situation, *and* a situation obtainable with no control. Picture B represents high concentrations of mosquitoes that would occur with light control activities. Picture C represents moderate concentrations of mosquitoes that would occur with moderate control activities. Picture D represents light concentrations that would occur with high control activities. As the pictures show, one can easily determine the concentration of mosquitoes. Please note that a picture depicting zero mosquitoes during the mosquito season is not obtainable by *any* mosquito control agency based on the available control tools, numerous breeding sites, and the breeding cycle of mosquitoes.

[4] A variety of visual aids and cards used in the survey to help define the good as well as questions designed for respondents that bid zero to establish reasons for zero bids are available upon request from the authors.

These relative concentration levels were developed with the assistance of the Chatham County Mosquito Control Commission (CCMCC) director, so as to correspond to light-trap data and landing rate counts associated with "light," "moderate," "high," and "outbreak" concentration levels. The photos were meant to represent a situation where an individual remaining stationary would experience the depicted level of mosquitoes over the course of a minute under daylight conditions. Although some researchers may consider these situations to be arbitrary and/or subjective, the relative concentration levels were developed as objective as current data allow, based on light-trap, landing count data and adult density index measures used by some MCDs along with expertise of CCMCC personnel knowledgeable of local residents' threshold/tolerance levels.

This was followed by a question designed to have the respondent distinguish among the concentration levels accompanied by another visual aid, the mosquito concentration ladder which anchors the photos on a 0–10 scale:

> B–1.a. Now, think about the level of mosquito concentrations in your county as a whole. HAND RESPONDENT CARD 5, "MOSQUITO CONCENTRATION LADDER." In terms of this scale from zero to ten, how would you rate the level of mosquito concentrations in your county at present time? POINT TO THE ZERO-TO-TEN SCALE AND CIRCLE NUMBER.
>
> 00 01 02 03 04 05 06 07 08 09 10 (GO TO B–1.b) UNSURE ..... 11 (GO TO B-1.d)

The respondent was then asked to consider valuing mosquito control with further description of the hypothetical market and payment mode (taxes):

> B–1.d. Now, we would like you to think about mosquito control in your county and the cost of this control. As a taxpayer, you pay directly for mosquito control in your county through tax dollars each year. The following questions are designed to measure how you value mosquito control.
>
> These questions concern the amount of money you would be willing to pay each year for various levels of mosquito control in only your county. Please keep in mind that the amounts you would pay each year would be paid in the form of higher taxes. In addition, consider the activities, both recreational and everyday activities, that you *presently* do, and that you *might* do in the future, in which mosquitoes now cause, and could cause some discomfort or nuisance to you. In other words, in the absence of mosquitoes, these present and future activities would probably be more enjoyable to you.

Tax payments were chosen because it is how most mosquito control programs are supported and, hence, adds to the realism of the overall benefit assessment. However, it should be noted that researchers question the use of taxes as a payment mode because of negative reactions sometimes elicited (Schulze et al. 1981, Mitchell and Carson 1981, Desvousges et al. 1983). As an alternative some researchers have used contributions to a fund specifically for the good in question (Walsh et al. 1984, Loehman 1984).

The next set of questions involved the valuation procedure, but, first a reference point was established to place all survey respondents at the same position. This was accomplished by having the respondent consider a situation of *no* mosquito control in their locality:

> Imagine that all current funding for mosquito control in your county were to *stop,* so that your county *no longer* had a mosquito control program. Picture A would represent this situation showing the amount of mosquitoes that would be present. One way to think of this would be that you could *expect* this level of mosquitoes to occur throughout a normal mosquito season in your county *without* a control program. In addition, Picture A represents a situation where the threat of disease transmission by mosquitoes is at its highest, as will be the nuisance aspect of mosquitoes.

This was followed by the valuation question, a direct WTP question accompanied by a payment card:

> 2–1. This payment card shows different yearly amounts people might be willing to pay for different levels of mosquito control and mosquito concentrations. HAND RESPONDENT CARD 6-A, "PAYMENT CARD," AND ALLOW RESPONDENT TIME TO LOOK AT IT.
>
> Considering that you would be faced with living in your county *without* a mosquito control program, the *high* public health threat, the *high* nuisance aspect, *and* the fact that to provide mosquito control in your county costs money; if a public body such as the county commission were to establish a mosquito control agency to provide control for your county:

What is the most it is worth to you (and your family) each year in higher taxes to *establish* a mosquito control agency in your county that could provide control, so that the situation represented by Picture B is achieved? Please pick any amount on the card, any amount in between, or any amount you think is appropriate.

$_____/year.

IF ANY AMOUNT, GO TO 2–2.

IF ZERO DOLLARS, ASK: Would it be worth something to you (and your family) to *establish* a mosquito control agency in your county capable of reducing the level of mosquitoes from Picture A to a lower level? CIRCLE NUMBER.

YES..... 01 (GO TO 2–2)
NO ..... 02 (GO TO 2–4)

If respondents indicated a nonzero amount they advanced to the next valuation question while respondents that bid zero were asked if they would bid a nonzero amount if a lower level of mosquito concentrations were achieved. If "yes" they advanced to the next valuation question. For those respondents not willing to pay more than zero they were asked whether they would change their outdoor activity patterns if faced with a situation of *no* control and a series of other questions to help establish a reason.

Sequential valuation questions were asked corresponding to the C and D photos:

2–2. (In addition to the amount you just told me), What is the most it is worth to you (and your family) each year in higher taxes to *establish* a mosquito control agency in your county that could provide control, so that the situation represented by Picture C is achieved?

$_____/year.

2–3. (In addition to the amount you just told me), What is the most it is worth to you (and your family) each year in higher taxes to *establish* a mosquito control agency in your county that could provide control, so that the situation represented by Picture D is achieved?

$_____/year.

APPLICATION. Time and funding limitations prevented a full scale application. The data were obtained from participation of Chatham County Mosquito Control Commission (CCMCC) and Glynn County Mosquito Control Commission (GCMCC) personnel. This application serves primarily to demonstrate the approach outlined above rather than obtaining representative benefit estimates of the general public. Two issues must be addressed concerning the relative size of the sample which involves properties of the estimators, and the representativeness of this sample involving sampling properties. Too few sample units drawn from a population may not accurately represent the population and could lead to biased estimates of the parameters in question. The point here concerns the variance about the population parameter (e.g., benefit estimates of control). Because the estimate of the variance of a population parameter is influenced by sample size, a small sample size will very likely result in a large estimated variance. In our discussion below we show that our benefit estimates are at least no more variable than benefit estimates in studies based on CMV approaches. Next we consider the representativeness of these individuals relative to the general public. Improper selection of the sample units could cause the sample to be unrepresentative of the population resulting in a sampling bias. Because one would expect these individuals to be more knowledgeable about mosquito control practices than the general public, whether or not this would result in more conservative benefit estimates is the crux of the issue. Unfortunately this cannot be formally examined with our data but is left for future endeavors. On the basis of education and income levels these individuals represent a fairly wide cross-section. Based on the overall conduct of the survey and rapport with these individuals, we believe these estimates may slightly understate benefits of mosquito control. In spite of any of the above shortcomings of these data we feel that these results merit reporting.

Estimates of annual benefits from improved levels of mosquito control on a per household and per capita (per person) basis are respectively presented in Tables 1 and 2. Concerning benefits associated with the incremental steps (marginal WTP)—outbreak to heavy, heavy to moderate, and moderate to light concentration levels—excluding influential observations,[5] incremental benefits were largest for the improvement from outbreak to heavy concentrations (or no control to light control activity). Mean estimates were $31.09/household or $9.47/person in 1984. Improvements in control associated with reducing mosquito concentrations from moderate to light levels contained the next highest benefit estimates.

Cumulative benefits associated with outbreak to moderate, outbreak to light, and heavy to

[5] Influential observations sometimes referred to as outliers can cause a problem with nonmarket good valuation studies. The techniques used to identify influential observations are based on Belsley, Kuh and Welsch (1980) and have been used before in nonmarket valuation studies (Desvousges et al. 1983, Mitchell and Carson 1984).

Table 1. Annual Benefit Estimates for Mosquito Control Improvements, 1984

	Outbreak to Heavy		Heavy to Moderate		Moderate to Light		Outbreak to Moderate		Outbreak to Light		Heavy to Light	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
	$/HH											
Full Data Set:												
Chatham	78.45 ± 169.64	22	66.86 ± 169.95	22	78.64 ± 169.91	22	145.32 ± 337.97	22	223.95 ± 503.81	22	145.50 ± 335.85	22
Glynn	23.08 ± 18.43	13	10.85 ± 9.85	13	11.31 ± 10.52	13	33.92 ± 27.12	13	45.23 ± 32.91	13	22.15 ± 18.05	13
Both	57.89 ± 136.49	35	46.06 ± 136.48	35	53.63 ± 137.70	35	103.94 ± 271.65	35	157.57 ± 406.00	35	99.69 ± 271.00	35
Data Set Without Outliers:[a]												
Chatham	44.10 ± 54.26	21	31.95 ± 46.61	21	44.29 ± 55.29	21	76.05 ± 95.35	21	120.33 ± 135.95	21	76.24 ± 87.29	21
Glynn	23.08 ± 18.43	13	10.85 ± 9.85	13	11.31 ± 10.52	13	33.92 ± 27.12	13	45.23 ± 32.91	13	22.15 ± 18.05	13
Both	36.06 ± 44.89	34	23.88 ± 38.21	34	31.68 ± 46.45	34	59.94 ± 78.80	34	91.62 ± 113.88	34	55.56 ± 73.81	34
Data Set Without Outliers:[b]												
Chatham	36.30 ± 41.90	20	23.55 ± 26.94	20	36.50 ± 43.34	20	59.85 ± 61.40	20	90.89 ± 99.10	19	56.63 ± 63.63	19
Glynn	23.08 ± 18.43	13	10.85 ± 9.85	13	11.31 ± 10.52	13	33.92 ± 27.12	13	45.23 ± 32.91	13	22.15 ± 18.05	13
Both	31.09 ± 34.83	33	18.55 ± 22.52	33	26.58 ± 36.24	33	49.64 ± 51.77	33	72.34 ± 81.49	32	42.63 ± 52.66	32
Outliers:												
Chatham	500.00 ± 424.26	2	500.00 ± 424.26	2	500.00 ± 424.26	2	1000.00 ± 848.53	2	1066.67 ± 1159.02	3	708.33 ± 773.12	3

Note: HH refers to households.
a. Corresponds to the data set where influential observations with WTP bids in excess of $200/HH are deleted from analysis.
b. Corresponds to the data set where influential observations with WTP bids in excess of and equal to $200/HH are deleted from analysis.

light concentration levels were also estimated. Average per capita benefits corresponding to these respective levels were estimated at $16.31/person, $24.17/person, and $14.87/person in 1984.

Comparing the relative variability (SD/$\bar{X}$) of these estimates with some recent CMV studies indicates that these mosquito benefit estimates ranged from 1.1 to 1.5 ($/person) and 1.0 to 1.4 ($/HH). In a study assessing benefits of improved water quality (Desvousges et al. 1983), relative variability of user values ranged from 1.8 to 2.5 (user only) and 3.5 to 4.6 (user and nonuser combined). Loehman (1984) examined the benefits of improved air quality and ranges of the relative variability of benefit estimates range from 1.2 to 1.5, 1.1 to 1.6, 1.2 to 1.3, and 1.6 depending on the initial quality level respondents started with. It appears that our benefit estimates demonstrate a similar degree of variability compared to the above studies, although a larger sample might have produced less variable benefit estimates (the Desvousges et al. study was based on a sample of 17—user, 54—user and nonuser; the Loehman study a sample of 145, 64, 83 and 43 respondents corresponding to the above ranges).

When asked about how these respondents felt about their "bids," 43% indicated that their answers were "quite accurate," and 43% accurate in a "ball park sense." In addition, 89% of the survey respondents felt that their benefit estimates were at least a good guide in valuing

Table 2. Annual Per Capita Benefit Estimates for Mosquito Control Improvements, 1984

	Outbreak to Heavy		Heavy to Moderate		Moderate to Light		Outbreak to Moderate		Outbreak to Light		Heavy to Light	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
	$/person											
Full Data Set:												
Chatham	30.27 ± 83.88	22	28.35 ± 84.31	22	30.13 ± 83.77	22	58.62 ± 167.89	22	88.75 ± 251.21	22	58.48 ± 167.61	22
Glynn	7.43 ± 5.57	13	4.00 ± 3.58	13	4.41 ± 5.34	13	11.43 ± 8.46	13	15.84 ± 11.83	13	8.41 ± 8.18	13
Both	21.78 ± 66.94	35	19.31 ± 67.36	35	20.58 ± 67.11	35	41.09 ± 134.05	35	61.67 ± 200.76	35	39.89 ± 134.08	35
Data Set Without Outliers:[a]												
Chatham	12.66 ± 15.04	21	10.65 ± 15.14	21	12.52 ± 14.23	21	23.31 ± 28.37	21	35.83 ± 39.70	21	23.17 ± 26.37	21
Glynn	7.43 ± 5.57	13	4.00 ± 3.58	13	4.41 ± 5.34	13	11.43 ± 8.46	13	15.84 ± 11.83	13	8.41 ± 8.18	13
Both	10.66 ± 12.45	34	8.11 ± 12.42	34	9.42 ± 12.21	34	18.77 ± 23.42	34	28.19 ± 33.22	34	17.53 ± 22.33	34
Data Set Without Outliers:[b]												
Chatham	10.79 ± 12.69	20	8.69 ± 12.48	20	11.15 ± 13.10	20	19.48 ± 22.86	20	29.87 ± 35.21	19	19.30 ± 24.10	19
Glynn	7.43 ± 5.57	13	4.00 ± 3.58	13	4.41 ± 5.34	13	11.43 ± 8.46	13	15.84 ± 11.83	13	8.41 ± 8.18	13
Both	9.47 ± 10.49	33	6.84 ± 10.13	33	8.49 ± 11.12	33	16.31 ± 18.79	33	24.17 ± 28.69	32	14.87 ± 19.82	32
Outliers:												
Chatham	225.00 ± 247.49	2	225.00 ± 247.49	2	200.00 ± 254.56	2	450.00 ± 494.97	2	461.67 ± 640.24	3	306.67 ± 427.50	3

Note: HH refers to households.
a. Corresponds to the data set where influential observations with WTP bids in excess of $200/HH are deleted from analysis.
b. Corresponds to the data set where influential observations with WTP bids in excess of and equal to $200/HH are deleted from analysis.

Table 3. Socioeconomic Characteristics, 1984

	Education		Income		Age		Household Size		Leisure Time	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
	- - - years - - -		- - - - $ - - - -		- - - - years - -		- - - - - no. - - -		- - hrs/week - - -	
Full Data Set:										
Chatham	13.3 ± 2.5	22	23,452 ± 14,285	21	42.5 ± 14.0	22	3.1 ± 1.4	22	22.4 ± 13.8	22
Glynn	12.5 ± 1.8	13	17,884 ± 16,261	13	32.8 ± 14.2	13	3.3 ± 1.3	13	22.0 ± 14.2	13
Both	13.0 ± 2.3	35	21,323 ± 15,078	34	38.9 ± 14.7	35	3.2 ± 1.3	35	22.3 ± 13.7	35
Data Set Without Outliers:[a]										
Chatham	13.3 ± 2.6	21	22,999 ± 14,500	20	41.7 ± 13.7	21	3.2 ± 1.4	21	22.9 ± 13.9	21
Glynn	12.5 ± 1.8	13	17,884 ± 16,261	13	32.8 ± 14.2	13	3.3 ± 1.3	13	22.0 ± 14.2	13
Both	13.0 ± 2.3	34	20,984 ± 15,180	33	38.3 ± 14.4	34	3.2 ± 1.3	34	22.6 ± 13.8	34
Data Set Without Outliers:[b]										
Chatham	13.4 ± 2.6	20	22,763 ± 14,858	19	41.7 ± 14.1	20	3.2 ± 1.4	20	22.9 ± 14.3	20
Glynn	12.5 ± 1.8	13	17,884 ± 16,261	13	32.8 ± 14.2	13	3.3 ± 1.3	13	22.0 ± 14.2	13
Both	13.0 ± 2.4	33	20,781 ± 15,377	32	38.2 ± 14.6	33	3.2 ± 1.3	33	22.5 ± 14.0	33
Data Set Without Outliers:[c]										
Chatham	13.4 ± 2.6	20	22,763 ± 14,858	19	41.7 ± 14.1	20	3.1 ± 1.4	20	23.8 ± 13.7	20
Glynn	12.5 ± 1.8	13	17,884 ± 16,261	13	32.8 ± 14.2	13	3.3 ± 1.3	13	22.0 ± 14.2	13
Both	13.0 ± 2.4	33	20,781 ± 15,377	32	38.2 ± 14.6	33	3.2 ± 1.3	33	23.1 ± 13.7	33
Data Set Without Outliers:[d]										
Chatham	13.4 ± 2.7	19	22,499 ± 15,243	18	41.8 ± 14.5	19	3.1 ± 1.4	19	23.7 ± 14.1	19
Glynn	12.5 ± 1.8	13	17,884 ± 16,261	13	32.8 ± 14.2	13	3.3 ± 1.3	13	22.0 ± 14.2	13
Both	13.0 ± 2.4	32	20,564 ± 15,582	31	38.1 ± 14.8	32	3.2 ± 1.3	32	23.0 ± 13.9	32
Outliers:										
Chatham[b]	13.0 ± 1.4	2	29,999 ± 3,536	2	50.5 ± 14.1	2	3.0 ± 1.4	2	18.0 ± 8.5	2
Chatham[c]	13.0 ± 1.4	2	29,999 ± 3,536	2	50.5 ± 14.1	2	3.5 ± 2.1	2	9.0 ± 4.2	2
Chatham[d]	12.7 ± 1.2	3	29,166 ± 2,887	3	47.2 ± 11.5	3	3.7 ± 1.5	3	14.0 ± 9.2	3

mosquito control (54%—fairly good guide, 34%—a good guide).

Various socioeconomic characteristics associated with these individuals are in Table 3.[6] Inclusion of this profile information will allow comparisons with other studies and further examination of the representativeness of these individuals with the general public. Examining the data set that corresponds to the benefit estimates above (data set without outliers for outbreak to light, and heavy to light levels) the average age of these individuals was 38 years, along with an average education of 13 years. Mean income was $20,564/year per household, and the average household size was 3.2 persons. Other information of interest to us was the amount of leisure time available—average of 23 hrs/week, the number of outdoor activities these individuals participate in—mean of 6.7 activities (e.g., gardening/yardwork, cookouts/picnics, porch-patio sitting, fishing—salt and fresh water, and hiking—five most popular in which mosquitoes are bothersome in descending order of importance), and the relative level of mosquito concentrations at the time of the survey (September 1984) ranked on a 0 to 10 scale—mean 5.8 ± 2.6 which corresponds to a relative density represented by Photo C, moderate concentrations.

Various attitude-type questions were asked about mosquito control perceptions. Highlights follow. An overwhelming majority (94%) felt that mosquito control was essential in these counties. Ninety-seven percent of the respondents dismissed the suggestion that mosquito control is an unnecessary luxury, 66% would favor a tax increase to ensure mosquito control, 77% disagreed with the statement "I can live with mosquitoes," 80% indicated they would like to see an expansion of mosquito control efforts in adjacent coastal areas (presently without control), 89% agreed that knowing the value of mosquito control can help make better decisions about resource allocation, and 86% felt that this kind of research is a good idea.

DISCUSSION

When faced with goods characterized by an absence of market conditions the researcher has little choice but to adopt alternative approaches to assist in quantifying and valuing these goods. The contingent market valuation (CMV) approach based on the willingness-to-pay concept is but one approach.

[6] Supplementary tables about socioeconomic distribution of the survey respondents, attitudes about mosquito control, and relative importance of insect biting pests are available upon request from the authors.

Table 3, continued

	Outdoor Activities		Race[e]		Mosquito Concentration Rating[f]		Length of Residence	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
	- - - no. - -						- - - - years - -	
Full Data Set:								
Chatham	6.1 ± 3.3	22	0.59 ± 0.50	22	6.2 ± 3.0	21	14.2 ± 10.9	12
Glynn	7.7 ± 2.7	13	0.69 ± 0.48	13	5.5 ± 1.8	13	7.2 ± 4.1	5
Both	6.7 ± 3.1	35	0.63 ± 0.49	35	5.9 ± 2.6	34	12.1 ± 9.8	17
Data Set Without Outliers:[a]								
Chatham	6.0 ± 3.4	21	0.57 ± 0.51	21	6.2 ± 3.0	20	14.2 ± 10.9	12
Glynn	7.7 ± 2.7	13	0.69 ± 0.48	13	5.5 ± 1.8	13	7.2 ± 4.1	5
Both	6.7 ± 3.2	34	0.62 ± 0.49	34	5.9 ± 2.6	33	12.1 ± 9.8	17
Data Set Without Outliers:[b]								
Chatham	6.1 ± 3.5	20	0.60 ± 0.50	20	6.0 ± 3.0	19	14.2 ± 10.9	12
Glynn	7.7 ± 2.7	13	0.69 ± 0.48	13	5.5 ± 1.8	13	7.2 ± 4.1	5
Both	6.7 ± 3.2	33	0.64 ± 0.49	33	5.8 ± 2.6	32	12.1 ± 9.8	17
Data Set Without Outliers:[c]								
Chatham	5.9 ± 3.4	20	0.55 ± 0.51	20	6.2 ± 3.1	19	14.2 ± 10.9	12
Glynn	7.7 ± 2.7	13	0.69 ± 0.48	13	5.5 ± 1.8	13	7.2 ± 4.1	5
Both	6.6 ± 3.2	33	0.61 ± 0.50	33	5.9 ± 2.6	32	12.1 ± 9.8	17
Data Set Without Outliers:[d]								
Chatham	5.9 ± 3.5	19	0.58 ± 0.51	19	6.0 ± 3.1	18	14.2 ± 10.9	12
Glynn	7.7 ± 2.7	13	0.69 ± 0.48	13	5.5 ± 1.8	13	7.2 ± 4.1	5
Both	6.7 ± 3.2	32	0.63 ± 0.49	32	5.8 ± 2.6	31	12.1 ± 9.8	17
Outliers:								
Chatham[b]	6.0 ± 1.4	2	0.50 ± 0.71	2	8.5 ± 0.7	2	-	-
Chatham[c]	8.0 ± 1.4	2	1.00 ± 0.00	2	7.0 ± 1.4	2	-	-
Chatham[d]	7.0 ± 2.0	3	0.67 ± 0.58	3	7.7 ± 1.5	3	-	-

a. Corresponds to the data set where influential observations with WTP bids in excess of $200/HH are deleted from analysis.
b. Corresponds to the data set where influential observations with WTP bids in excess and equal to $200/HH associated with improvements from outbreak to heavy (photo B) and heavy to moderate (photo C) levels as well as total bid for outbreak to moderate (photos B + C) levels are deleted from analysis. Outliers are simply the observations deleted.
c. Corresponds to the data set where influential observations with WTP bids in excess and equal to $200/HH associated with moderate to light levels only, are deleted from analysis. Outliers are simply the observations deleted.
d. Corresponds to the data set where influential observations with WTP bids in excess and equal to $200/HH associated with outbreak to light (photos B + D) and heavy to light (photos C + D) levels are deleted from analysis. Outliers are simply the observations deleted.
e. 1 if white, 0 if nonwhite.
f. 0 = lowest, 10 = highest.

This paper develops a CMV approach that can be used to value public mosquito control. The preliminary study demonstrates how this approach can be used to assess and interpret a value of benefits from mosquito control. Compared to other CMV studies our benefit estimates demonstrate a similar degree of variability. With slight adaptations a similar procedure could be applied in different areas; however, we recommend that researchers not familiar with nonmarket good benefit techniques seek professional assistance from economists knowledgeable about these techniques.

The CMV approach is composed of four critical interrelated elements, the hypothetical market, the good to be valued, the payment mode, and the valuation questions. Much care must be taken in advancing both the good and market setting in the questionnaire design so that they appear realistic and credible, and are easily understood by the survey respondent. The valuation questions must not only be unambiguous but also must appear credible to the respondent. All of these elements are intertwined and of equal importance in questionnaire design. Carelessness in any of these

parts would not only cast doubt on the survey application, but, add to the skepticism associated with the CMV approach and results. As with any study based on survey data the study is only as good as its data and this begins with the design of the survey instrument along with the sampling method.

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Human exposure to insecticide products containing pyrethrins and piperonyl butoxide (2001–2003)

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ABSTRACT

Pyrethrum, used as an insecticide for centuries, is derived from dried and ground flowers of *Chrysanthemum cinerariaefolium*. Its current major use is in insecticide products to the control insects in the home and food handling establishments. We investigated human incidents reported through the American Association of Poison Control Centers (AAPCC) Toxic Exposure Surveillance System (TESS©) associated with regulated insecticides containing pyrethrins and piperonyl butoxide (PY/PBO) from 2001 to 2003. Special attention was paid to dermal and respiratory effects. Although there are limitations associated with TESS data, we observed that

- Despite extensive use, incidents with reports of moderate or major adverse effects were relatively rare (717 moderate and 23 major outcomes out of 17,873 calls).
- Following label-directed use of the products, adverse dermal or respiratory reactions were very rare; (dermal – 17 moderate, 1 major; respiratory – 18 moderate, 0 major).
- The data suggest that asthmatics and people sensitive to ragweed (*Ambrosia artisifolia*) are not unusually sensitive to PY/PBO.

In view of their widespread use, the data indicates that PY/PBO products can be used with a relatively low risk of adverse effects. Moreover, the data suggest that they are not likely to cause reactions in people with asthma or allergies.

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1. Introduction

Assessment of the risk of an active ingredient to humans requires careful consideration of the animal toxicology data as well as the human use experience. The purpose of this report is to investigate the human incident reports associated with the use of EPA-regulated insecticide products containing pyrethrins and piperonyl butoxide (PY/PBO) from 2001 to 2003. Special attention will be paid to dermal and respiratory effects. Our focus will be on data collected as part of the Toxic Exposure Surveillance System (TESS©) managed by the American Association of Poison Control Centers (AAPCC).

1.1. Background

Pyrethrum, used as an insecticide for centuries, is derived from dried and ground flowers of *Chrysanthemum cinerariaefolium* which is a member of the Compositae family. It is presently used in insecticide products for the control of crawling and flying insects in the home and food handling establishments, to a limited degree in agriculture, and in medicine to control lice on humans. Pyrethrins (PY) are the insecticidally active ingredients in pyrethrum extract. They are categorized into two groups Pyrethrins I (pyrethrin 1, cinerin 1, jasmolin 1) and Pyrethrins II (pyrethrin 2, cinerin 2, jasmolin 2). Pyrethrins (CAS #8003-34-7) is the term used on most current product labels to describe Pyrethrin I and Pyrethrin II collectively, also known as Total Pyrethrins. Detailed reviews of the toxicology of pyrethrins have been conducted by the World Health Organization/Food and Agriculture Organization (WHO/FAO) Joint Meetings on Pesticide Residues (JMPR, 1999, 2003) .

Pyrethrum has been reported to produce allergic contact dermatitis in humans (Osimitz et al., 2006). Moreover, it has been speculated that cross reactions occur in people sensitized to ragweed (*Ambrosia artisifolia*). A recent evidence-based review of the

Abbreviations: AAPCC, American Association of Poison Control Centers; CDC, Centers for Disease Control; EPA, Environmental Protection Agency; FDA, Food and Drug Administration; NHANES, National Health and Nutritional Examination Surveys; NHIS, National Health Interview Surveys; PBO, piperonyl butoxide; PY, Pyrethrins; SPI, Specialist in Poison Information; TESS©, Toxic Exposure Surveillance System.

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botany, contemporary chemistry and case reports of alleged allergic contact dermatitis (Osimitz et al., 2006) concluded that the evidence does not show that allergic contact dermatitis, or cross reactions in ragweed sensitized people results from exposure to the pyrethrins. It is important to note that the authors report that today's refined extracts (since 1967) have lower levels of the putative dermal sensitizer as compared to either ground pyrethrum flowers or the extracts used earlier in the 20th century.

An additional evidenced-based review of literature failed to reveal a clear causal association between pyrethrins and contact urticaria (Type I hypersensitivity) (Franzosa et al., 2007).

Pyrethrins have also been claimed to be associated with adverse respiratory responses (Carlson and Villaveces, 1977; Culver et al., 1988; Garratt and Bigger, 1923; Lewis, 1991; Newton and Breslin, 1983; Paton and Walker, 1988; Ramirez, 1930; Wagner, 1994, 2000; Wax and Hoffman, 1994; Zucker, 1965). Although details suggesting pyrethrins are specific inducers of respiratory effects are lacking, it is possible that some pyrethrins-containing products or non-insecticidal components of the product formulation may be acting as non-specific irritants resulting in adverse respiratory effects.

Piperonyl butoxide (PBO, CAS 51-03-6) is a synergist that inhibits the mixed function oxidase system, thereby slowing the oxidative breakdown of other pesticides like pyrethrins and the synthetic pyrethroids. It appears in over 1000 pesticide registrations in the US, mostly along with pyrethrins. It is generally of low toxicity and has not been specifically associated with dermal or respiratory reactions (Osimitz and Breathnach, 2001).

1.2. TESS and rational for use in examining pyrethrins/PBO incidents

The primary purpose of the Poison Control Center system in the United States is to provide patient-specific emergency assessment and treatment information to callers, and to triage them to a medical facility for examination and further treatment if the reported exposure and symptoms suggest this is necessary. The TESS dataset contains data fields categorizing information obtained from callers to poison centers about the substance involved in an exposure, age and sex of the person exposed, reason for exposure (accidental, intentional, etc.), site of exposure, duration of exposure (acute, chronic, etc.), route of exposure (inhalation, ingestion, etc.), patient management and treatment (treated on site, treated and released, admitted for medical or psychiatric care, etc.), symptoms reported and medical outcome. Medical outcome is a rating of the severity of the effects made by the Specialist in Poison Information (SPI).

To better understand the likelihood of pyrethrins/PBO exposure to result in either dermal or respiratory effects, exposures reported through TESS were selected for review. Several aspects of TESS changed around 2000, including the separation of pyrethrins from the pyrethroids and the elimination of the previous false distinction between pyrethrins/PBO and pyrethrins alone products. Thus, pyrethrins and pyrethrins/PBO products were not distinguished in the 2001–2003 dataset selected for this report.

A total of 58,178 calls were reported to TESS on insecticide products containing pyrethrins, pyrethrins/PBO, or the synthetic pyrethroids from 2001 to 2003. Pyrethrins/PBO accounted for 20,511 of those calls.

Despite limitations associated with this and other systems of spontaneously reported adverse incident data, the TESS dataset, with its standardized data fields and methods of documenting and classifying incidents, was selected as the most appropriate adverse incident dataset available to learn about possible adverse health effects potentially associated with consumer products.

Because the intent of this analysis is to learn about the safety of PY/PBO in EPA-regulated insecticides, calls relating to Food and Drug Administration (FDA)-regulated pediculicide use (intentional administration to the skin) were excluded. Thus, the knowledge developed from our investigation may or may not be applicable to such direct uses.

It is essential to keep in mind that any examination of the number and nature of reported adverse effects potentially associated with a consumer product must be considered in the context of the extent of product use during the study period. Sales of household insecticides containing PY/PBO during the period of this review (2001–2003) are estimated to be over 40,000,000 individual product units (S.C. Johnson and Son, Inc., personal communications, 2008). If one assumes that a unit is used on four occasions, this suggests that over 160,000,000 uses of PY/PBO products occurred during the review period. Given that many households have more than one person in them, it is reasonable to assume that over 300,000,000 people may have had some exposure to PY/PBO products over the 3 years of the study period.

2. Materials and methods

2.1. Data used for this analysis

The electronic records of all reported human exposures represented in the TESS dataset to products generically classified as containing pyrethrins and/or PBO from 2001 to 2003 were purchased from the AAPCC. Additionally, only exposures where a single product was involved were included in the analysis as multiple substance exposures confound the understanding of the exposure experience related to pyrethrins/PBO. Data were provided in a Microsoft Access format for retrieval of data subsets.

Although tabulation of incidents included all pyrethrins/PBO exposures regardless of effect, the focus was placed on those incidents reporting potential dermal and respiratory irritation. Unfortunately, the TESS system does not contain a data field designated to capture allergy or pre-existing medical condition information of the caller, such as allergy status of hypersensitivities. Thus, a surrogate measure of association was selected. The treatment options documented in each case record were used to identify treatments typically associated with more intense respiratory or dermal effects. Records were searched for indication of bronchodilator use as part of the treatment as suggestive that simple fresh air or ventilation was insufficient to alleviate signs and symptoms likely associated with shortness of breath patients with pre-existing airway disease. With respect to dermal effects, steroid treatment as suggestive of a need for treatment involving more than simple irrigation or washing of the affected area was reviewed. Differentiation between oral versus topical steroid use was not possible with the available data.

3. Results

TESS data documents that from 2001 to 2003, 7,043,589 human exposure cases to chemicals and products of all kinds were reported by participating poison centers. The annual human exposure case volume by center ranged from 11,458 to 1,10,459 (mean 38,388) for centers participating for the entire year. Penetration, defined as the number of human poison exposure cases reported per 1000 individuals per year in the population served ranged from 5.9 to 17.6 with a mean of 8.1. A total of 15,427 calls to poison control centers were made with regard to PY/PBO containing products from 2001 to 2003.

3.1. Medical outcomes

The severity of the reported symptoms is recorded in the field called "medical outcome". The PSI at the AAPCC rates the severity of the case based on specific criteria as well as the overall relationship of clinical effects to the suspected toxicant. Unless the case is specifically coded as "unrelated", it is assumed that the reported effects and rated outcome severity are likely related to exposure to the listed chemical. Outcome severity and definitions are listed in Appendix A. Note that there are two categories of outcomes where SPI have the latitude to code an outcome with a reasonably predicted assessment of the likelihood of adverse effects. These comprise the "not-followed" outcomes where follow-up is unsuccessful or deemed unnecessary, yet an outcome is reasonably

Table 1
Summary of medical outcome. Medical outcomes – #(%).

Year	No effect[a]	Minor effect	Moderate effect	Major effect	Fatality	Case not followed, not toxic	Case not followed, no more than minor	Unable to follow, potentially toxic exposure	Unrelated effect	Unknown, field incomplete
2001	985 (18%)	1219 (22%)	229 (4%)	8 (0.1%)	0	476 (9%)	1917 (35%)	119 (2%)	498 (9%)	0
2002	906 (18%)	1141 (23%)	199 (4%)	9 (0.2%)	0	418 (8%)	1800 (36%)	105 (2%)	438 (9%)	1 (0%)
2003	837 (17%)	1149 (23%)	186 (4%)	2 (0.04%)	0	400 (8%)	1883 (38%)	93 (2%)	409 (8%)	0
Total	**2728 (17.7%)**	**3509 (22.7%)**	**614 (4%)**	**19 (0.1%)**	**0**	**1294 (8.4%)**	**5601 (36.3%)**	**317 (20.5%)**	**1345 (8.7%)**	**1**

[a] Although cases with symptoms are not intended to be coded "No Effect", occasionally some cases get that code.

predicted based on call characteristics, exposure scenarios and professional judgment of the specialist managing the case.

Table 1 shows the medical outcomes for all PY/PBO cases by year. Moderate or major outcomes were coded in 614 (4.0%) and 19 (0.1%) of the 15,427 calls received, respectively. No fatalities were recorded during the test period. In cases with known or reasonably predicted outcomes, 90.8% of cases resulted in either no symptoms or only minor symptoms.

3.2. Ages of callers and medical outcome by age

The age distribution of patients exposed to PY/PBO is shown in Table 2. Exposures were most common among adults with 8174 (53%) calls. There were also a significant number of calls involving children under the age of 6 years with 5377 (35%) calls despite children comprising less than 10% of the US population. This observation is consistent with general exposure trends representative of calls to poison centers where, historically, the majority of exposure calls involve children.

With regard to age and medical outcome, adults were the largest group reporting exposure to PY/PBO products. This group had a combined rate of minor and no effect outcomes of 79.6%. Despite having the second most frequent number of exposures, children younger than 6 years had little toxicity following exposure. It is noteworthy that 93% of the reported exposures involving this age group resulted in no adverse consequence and only 4/5377 (<0.1%) calls regarding children resulted in major outcomes.[1] Major outcomes were rare in all age groups.

3.3. Chronicity of the cases

Nearly all (97%) of the exposures were acute in nature. Only 1.5% were due to chronic use of a PY/PBO product.

3.4. Gender of the callers

Gender was unevenly split in the data with 55% of calls came from females (Table 3). This may reflect the fact that women tend to be the more frequent users of insect control products than men (S.C. Johnson and Son, Inc., personal communications, 2008).

3.5. Site of exposure

The site of the exposure was a residence in a high percentage of cases; own and other residences accounted for 94% of exposure sites (Table 4). Only 3.6% occurred at a workplace.

[1] These numbers only included cases where the medical outcome was classified as minor, no effect, not followed no effect, and not followed minimally toxic.

3.6. Management site

Management of PY/PBO cases was primarily onsite. Eighty-one percent of PY/PBO cases were managed at the site of the exposure, typically a residence. Twelve percent of calls originated while the patient was en route to a health care facility and, therefore, the patient was treated at that site. Patients were referred to a health care facility for assessment and treatment in approximately 6% of cases.

3.7. Reason for exposure

Within the TESS framework, exposure reasons are divided into the general categories of adverse reaction, intentional, unintentional and other. Specific definitions are included in Appendix B.

Cases coded as being adverse reactions are defined as resulting from routine and *appropriate label-directed use* of a product, as opposed to over use, misuse or abuse. Included in this classification are cases resulting in an unwanted effect due to an allergic, hypersensitivity or idiosyncratic response to the product. It is the adverse reactions that provide the best gauge of the relative safety of the product under label-directed conditions of use. Some cases, unfortunately, have too little information to accurately characterize the reason for exposure.

General and detailed descriptions of reasons for exposure are shown in Tables 5 and 6. Most exposures were classified as unintentional with approximately 11% classified as a misuse situation (either unintentional or intentional). Suicide and abuse exposures were uncommon at <1% each.

3.8. Route of exposure and associated medical outcome

Subjects were most commonly exposed to PY/PBO via ingestion (34.2%), inhalation/nasal (27.6%) and dermal (27.2%) exposure routes. Note that each individual case may include more than one route of exposure, thus, the total relative numbers of exposure routes and corresponding outcomes in this classification is greater than the total number of incidents.

Medical outcomes based on route of exposure are shown in Table 7. Cases may be coded as followed to a "known" outcome (no effect, minor, moderate, major, or death) or an assumed or reasonably predicted "not-followed" outcome ("not followed nontoxic", "not followed no more than minor effect possible") or an unknown but potentially toxic outcome "unable to follow potentially toxic effect possible"). The most common outcome was a not-followed case with no more than minor effect possible. Of those cases where outcome was confirmed with a follow-up phone call, minor effect was the most common outcome at 23.4%. When combined, minor effect cases or cases predicted as not more than minor effect, accounted for 59.4% of outcomes. Taking into account nontoxic and assumed nontoxic exposures, approximately 95% of

Table 2
Age distribution of medical outcome.

Age	Total	No effect[a]		Minor effect		Moderate effect		Major effect		Fatality		Not followed nontoxic		Not followed no more than minor		Unable to follow, potentially toxic		Unrelated effect		Total	
		n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Under 6	5377	1576	29.3	736	13.7	67	1.2	4	0.1	0	0.0	668	12.4	2021	37.6	62	1.2	243	4.5	5377	**34.9**
6–12 years	1071	196	18.3	249	23.2	26	2.4	1	0.1	0	0.0	106	9.9	386	36.0	10	0.9	97	9.1	1071	**6.9**
13–19 years	632	79	12.5	174	27.5	32	5.1	1	0.2	0	0.0	40	6.3	253	40.0	12	1.9	41	6.5	632	**4.1**
>19 years	8174	853	10.4	2307	28.2	488	6.0	13	0.2	0	0.0	465	5.7	2880	35.2	217	2.7	951	11.6	8174	**53.0**
Unknown	173	24	13.9	43	24.9	1	0.6	0	0.0	0	0.0	15	8.7	61	35.3	16	9.2	13	7.5	173	**1.1**
Total	**15,427**	**2728**	**17.7**	**3509**	**22.7**	**614**	**4.0**	**19**	**0.1**	**0**	**0.0**	**1294**	**8.4**	**5601**	**36.3**	**317**	**2.1**	**1345**	**8.7**	**15,427**	

[a] Although cases with symptoms are not intended to be coded "No Effect", occasionally some cases get that code.

Table 3
Gender.

Sex	Frequency	Percent
Male	6859	44
Female	8482	55
Unknown	86	0.60
Total	**15,427**	

Table 4
Exposure site.

Location	Frequency	Percent
Own residence	13,974	90.6
Other residence	526	3.4
Workplace	560	3.6
Health care facility	10	0.1
School	39	0.3
Restaurant/food service	15	0.1
Public area	143	0.9
Other	115	0.7
Unknown	45	0.3
Total	**15,427**	

Table 5
Exposure reasons – general.

Exposure reason	Frequency	Percent
Unintentional – any	14,565	94.4
Intentional – any	279	1.8
Adverse reaction	469	3.0
Other	88	0.6
Unknown reason	26	0.2
Total	**15,427**	

Table 6
Exposure reasons – detailed.

Exposure reason	Frequency	Percent
Unintentional – general	10,863	70.4
Unintentional – environmental	1510	9.8
Unintentional –occupational	367	2.4
Unintentional – therapeutic error	270	1.8
Unintentional – misuse	1503	9.7
Unintentional– bite/sting	1	0.0
Unintentional – food poisoning	10	0.1
Unintentional – unknown	41	0.3
Intentional – suspected suicide	69	0.4
Intentional – misuse	184	1.2
Intentional – abuse	11	0.1
Intentional – unknown	15	0.1
Other – contamination/tampering	39	0.3
Other – malicious	49	0.3
Adverse reaction – drug	56	0.4
Adverse reaction – food	2	0.0
Adverse reaction – other	411	2.7
Unknown reason	26	0.2
Total	**15,427**	

all cases were coded as minor or no effect. Further detail is shown in Table 7 describing the presence of any symptoms in patients who did not receive a follow-up phone call to confirm a predicted outcome.

In patients with confirmed outcomes, ingestion exposures were most likely to have no effect (Table 7). Callers exposed via inhalation and dermal routes had higher rates of minor outcomes than any

Table 7
Route of exposure and medial outcome.

Route of exposure	No effect[a]		Minor effect		Moderate effect		Major effect		Fatality		Not followed, nontoxic		Not followed no more than minor		Unable to follow, potentially toxic		Unrelated effect		Total	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Ingestion	1646	26.9	679	11.1	71	1.2	7	0.1	0	0.0	851	13.9	2450	40.1	73	1.2	332	5.4	6109	34.2
Inhalation/nasal	445	9.0	1463	29.7	381	7.7	11	0.2	0	0.0	159	3.2	1643	33.3	152	3.1	680	13.8	4934	27.6
Aspiration	1	16.7	3	50.0	2	33.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	0.0
Ocular	105	5.9	1018	57.0	94	5.3	3	0.2	0	0.0	22	1.2	464	26.0	63	3.5	17	1.0	1786	10.0
Dermal	958	19.7	995	20.4	163	3.3	2	0.0	0	0.0	371	7.6	1819	37.4	90	1.8	469	9.6	4867	27.2
Otic	4	9.8	2	4.9	1	2.4	0	0.0	0	0.0	7	17.1	25	61.0	2	4.9	0	0.0	41	0.2
Other	8	22.2	10	27.8	3	8.3	0	0.0	0	0.0	0	0.0	8	22.2	3	8.3	4	11.1	36	0.2
Unknown	15	16.0	13	13.8	2	2.1	0	0.0	0	0.0	7	7.4	21	22.3	1	1.1	35	37.2	94	0.5
Total	**3182**	**17.8**	**4183**	**23.4**	**717**	**4.0**	**23**	**0.1**	**0**	**0.0**	**1417**	**7.9**	**6430**	**36.0**	**384**	**2.1**	**1537**	**8.6**	**17,873**	

[a] Although cases with symptoms are not intended to be coded "No Effect", occasionally some cases get that code.

Table 8
Summary of symptoms by organ system.

Organ system	Frequency	Percent of total symptoms (%)	Percent of total cases (%)
Cardiovascular	240	2	2
Dermal	1814	13	12
Gastrointestinal	3508	26	23
Heme/hepatic	6	0	0
Neurological	1724	13	11
Ocular	2357	17	15
Renal	7	0	0
Respiratory	1925	14	12
Miscellaneous	1946	14	13
Total	**13,527**		

Table 9
Most common symptom by organ system.

Organ System	Symptom	Frequency	Percent of total cases (%)
Cardiovascular	Chest pain	143	0.9
Dermal	Irritation	626	4.1
Gastrointestinal	Vomiting	985	6.4
Neurological	Headache	634	4.1
Ocular	Irritation	1544	10.0
Respiratory	Cough/choke	1328	8.6

other. Moderate outcomes were seen in 381 (7.7% of all dermal calls) exposed via inhalation with 11 (0.2% of all dermal calls) experiencing major outcomes. Dermal exposure to PY/PBO resulted in moderate outcomes in 163 (5.3%) callers, but just 2 (<0.1% of all dermal calls) major outcome cases. Inhalation and dermal routes had a similar rate of nontoxic/minor effects compared to ingestions (range 38–40.1%) and rates of more serious outcomes (0.1% versus 0–0.2%).

3.9. Symptoms reported

Symptoms are categorized by organ system and specific description. The number of patients having any symptoms within an organ system is described in Table 8. Fewer than 3% of callers reported renal, hepatic or cardiovascular symptoms.

The most common symptoms by organ system are shown in Table 8. The most frequently reported symptoms were gastrointestinal. Ocular irritation was the most frequently reported symptom overall.

3.10. Callers with dermal symptoms

The percentage of patients with any dermal symptoms is 14% (Table 8). Dermal irritation was the most common dermal symptom (Table 9). Table 10 shows the result for callers reporting dermal symptoms regardless of reason for call (intentional or unintentional exposure, adverse effect). The duration of these symptoms is described in Table 11 (by age group). Dermal symptoms lasted for <24 h in 68% of cases.

In addition, Table 12 shows the number of patients receiving steroid treatment for their reactions. Treatment was more likely to be associated with symptoms; and patients without symptoms were less likely to receive a palliative or prophylactic treatment. Table 12 presents a further breakdown of the callers that received steroid treatment by reason for the call. These data show that serious dermal reactions (moderate or major outcomes) are rare and that only two patients experiencing an adverse reaction from a PY/PBO product received steroid therapy. Both of these cases were of moderate outcome severity.

Table 10
Medical outcomes – dermal symptoms. All exposure reasons (unintentional, intentional, and adverse reaction).

	No effect[a]		Minor Effect		Moderate effect		Major effect		Fatality		Not followed, nontoxic		Not followed minor		Unable to follow, potentially toxic		Total
	n	(%)	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	
Any dermal symptom	3	0.3	579	50.7	101	8.9	2	0.2	0	0.0	1	0.1	429	37.6	26	2.3	**1141**

[a] Although cases with symptoms are not intended to be coded "No Effect", occasionally some cases get that code.

Table 11
Clinical effects duration for dermal symptoms by age[a].

Age of patient	Clinical effects duration									
	⩽2 h	>2 h, ⩽8 h	> 8 h, ⩽24 h	>24 h, ⩽3 days	>3 days, ⩽1 week	>1 week, ⩽1 month	>1 month	Anticipated permanent	Unknown[b]	Total
Under 6	54	22	24	15	4	2	0	0	84	**205**
6–12 years	15	7	7	4	9	1	0	0	46	**89**
13–19 years	8	5	1	8	0	1	0	0	31	**54**
>19 years	87	89	87	68	40	19	16	1	473	**880**
Unknown	0	0	2	2	0	0	0	0	10	**14**
Total	**164**	**123**	**121**	**97**	**53**	**23**	**16**	**1**	**644**	**1242**[c]
% of all patients w/symptoms	13.2	9.9	9.7	7.8	4.3	1.9	1.3	0.1	51.9	
% of patients w/symptoms for a known duration	27.4	20.6	20.2	16.2	8.9	3.8	2.7	0.2	NA	598[d]

[a] Includes data from unintentional, intentional and adverse reaction cases only.
[b] If clinical effects duration is not coded, the data is recorded as an unknown duration.
[c] Total of symptoms (known and unknown durations).
[d] Total of symptoms (only unknown durations).

Table 12
Exposure reason, medical outcome, and treatment for dermal symptoms.

	Medical outcome																
	No effect		Minor effect		Moderate effect		Major effect		Fatality		Not followed, nontoxic		Not followed minor		Unable to follow, potentially toxic		Total
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	
Unintentional reason																	
Any dermal symptom	3	0.3	504	51.2	79	8.0	1	0.1	0	0.0	1	0.1	373	37.9	24	2.4	**985**
Any dermal symptom; treated with steroids	0	0.0	7	58.3	5	41.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**12**
Any dermal symptom; no steroid	3	0.3	497	51.1	74	7.6	1	0.1	0	0.0	1	0.1	373	38.3	24	2.5	**973**
Intentional																	
Any dermal symptom	0	0.0	14	60.9	3	13.0	0	0.0	0	0.0	0	0.0	6	26.1	0	0.0	**23**
Any dermal symptom; treated with steroids	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**0**
Any dermal symptom; no steroid	0	0.0	14	60.9	3	13.0	0	0.0	0	0.0	0	0.0	6	26.1	0	0.0	**23**
Adverse reactions																	
Any dermal symptom	0	0.0	61	45.9	19	14.3	1	0.8	0	0.0	0	0.0	50	37.6	2	1.5	**133**
Any dermal symptom; treated with steroids	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**2**
Any dermal symptom; no steroid	0	0.0	61	46.6	17	13.0	1	0.8	0	0.0	0	0.0	50	38.2	2	1.5	**131**

3.11. Callers with respiratory symptoms

Respiratory symptoms of any type were reported in 1925 cases (12% of all cases, Table 8). Cough/choke was the most common respiratory symptom (Table 9).

Table 13 shows the result for callers regardless of reason for call (intentional or unintentional exposure, adverse effect). The duration of these symptoms is described in Table 14 (by age group). Respiratory symptoms persisted for <24 h in approximately 87% of cases (Table 14).

In addition, Table 15 presents the number of callers receiving bronchodilator treatment for their reactions. Treatment was more likely to be associated with symptoms, as was the case with dermal symptoms. Patients without symptoms were less likely to receive a

Table 13
Medical outcomes – respiratory symptoms. All exposure reasons (unintentional, intentional, and adverse reaction).

	No effect[a]		Minor effect		Moderate effect		Major effect		Fatality		Not followed, nontoxic		Not followed minor		Unable to follow, potentially toxic		Total
	n	(%)	*n*	(%)	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	
Any respiratory symptom	0	0.0	733	47.4	311	20.1	8	0.5	0	0.0	0	0.0	424	27.4	71	4.6	1547

[a] Although cases with symptoms are not intended to be coded "No Effect", occasionally some cases get that code.

Table 14
Clinical effects duration for respiratory symptoms by age.

Age of patient	Clinical effects duration									Total
	⩽2 h	>2 h, ⩽8 h	>8 h, ⩽24 h	>24 h, ⩽3 days	>3 days, ⩽1 week	>1 week, ⩽1 month	>1 month	Anticipated permanent	Unknown[b]	
Under 6	90	13	11	6	1	1	0	0	52	**174**
6–12 years	36	9	4	1	1	0	0	0	35	**86**
13–19 years	32	13	7	3	1	0	0	1	30	**87**
>19 years	342	168	86	55	35	12	5	0	501	**1204**
Unknown	7	0	0	0	1	0	0	0	5	**13**
Total	**507**	**203**	**108**	**65**	**39**	**13**	**5**	**1**	**623**	**1564**[c]
% of all patients w/symptoms	32.4	13.0	6.9	4.2	2.5	0.8	0.3	0.1	39.8	
% of patients w/symptoms for a known duration	53.9	21.6	11.5	6.9	4.1	1.4	0.5	0.1	NA	**941**[d]

[a] Includes data from unintentional, intentional and adverse reaction cases only.
[b] If clinical effects duration is not coded, the data is recorded as an unknown duration.
[c] Total of symptoms (known and unknown durations).
[d] Total of symptoms (only unknown durations).

Table 15
Exposure reason, medical outcome, and treatment for respiratory symptoms.

	Medical outcomes																Total
	No effect		Minor effect		Moderate effect		Major effect		Fatality		Not followed, nontoxic		Not followed minor		Unable to follow, potentially toxic		
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	
Unintentional																	
Any respiratory symptom	0	0.0	686	47.1	288	19.8	8	0.5	0	0.0	0	0.0	406	27.9	67	4.6	**1455**
Any respiratory symptom; treated with bronchodilator	0	0.0	34	27.6	70	56.9	3	2.4	0	0.0	0	0.0	9	7.3	7	5.7	**123**
Any respiratory symptom; no bronchodilator	0	0.0	652	48.9	218	16.4	5	0.4	0	0.0	0	0.0	397	29.8	60	4.5	**1332**
Intentional																	
Any respiratory symptom	0	0.0	14	48.3	5	17.2	0	0.0	0	0.0	0	0.0	7	24.1	3	10.3	**29**
Any respiratory symptom; treated with bronchodilator	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**0**
Any respiratory symptom; no bronchodilator	0	0.0	14	48.3	5	17.2	0	0.0	0	0.0	0	0.0	7	24.1	3	10.3	**29**
Adverse reactions																	
Any respiratory symptom	0	0.0	33	52.4	18	28.6	0	0.0	0	0.0	0	0.0	11	17.5	1	1.6	**63**
Any respiratory symptom; treated with bronchodilator	0	0.0	4	40.0	6	60.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**10**
Any respiratory symptom; no bronchodilator	0	0.0	29	54.7	12	22.6	0	0.0	0	0.0	0	0.0	11	20.8	1	1.9	**53**

palliative or prophylactic treatment. A further breakdown of the callers that received bronchodilator treatment by reason for the call is also shown. The data show that serious respiratory reactions are rare with only 10 individuals experiencing an adverse reaction that required a bronchodilator for treatment. Of these 10, four had minor symptoms and six had a moderate outcome. No major (i.e. life-threatening) outcomes occurred.

4. Discussion

4.1. Limitations of TESS data

The TESS dataset is a valuable tool for understanding the human use experience with PY/PBO. However, despite its widespread use, this dataset has both strengths and weaknesses that in some cases limit or prohibit the drawing of firm conclusions regarding the safety of a given product or ingredient. These strengths and weaknesses have been previously reviewed extensively by Kingston et al. (1999). The following limitations should be kept in mind when evaluating the respiratory or dermal adverse event profile of PY/PBO products:

- Observational nature of TESS data – the vast majority of the calls are spontaneously reported incidents where consumer information is solicited and taken at face value without the benefit of independent confirmation by a medical provider.
- Lack of detailed narratives in the dataset – narrative descriptions are not part of the basic dataset, thus circumstances, corroborating medical findings or other useful information that would be helpful in further delineating the exposure is neither included nor determinable.
- No recording of pre-existing medical conditions – under TESS procedure, pre-existing conditions such as asthma and allergy are not tabulated for patients. Therefore, the likelihood that allergy or asthma contributed to any given effect in any specific case with any specific outcome is simply unknown. Additionally, the general symptom of "allergic" reaction is not a coded symptom field. What is coded, are symptoms that may or may not be related to an allergy or asthmatic response after exposure. These same symptoms may be the result of excessive and/or massive exposures.
- Lack of quantitative information on exposures – for example, all cases coded with an exposure route of inhalation/nasal or

dermal are considered equal in exposure rate and quantity. This is important when one considers the PY/PBO data, that as many as 94.5% of all cases were coded as "unintended". By definition, this is an exposure that was considered excessive or outside of the routine or labeled use pattern. Even when an unintended exposure occurs, there is no quantification of the exposure. Consider the case of a young child spraying a sibling in the face at close range for a full 30 s with a PY/PBO aerosol product as opposed to the same child spraying a brief burst of product in the direction of the sibling but 10 feet away. Both exposures are depicted identically in the dataset, but each represents a significantly different rate and quantity of exposure.

- Limited cause and effect information – the "medical outcome" field is often thought to confirm a "cause and effect" relationship between the suspected substance involved in the exposure and any reported adverse clinical effects. However, at best, the outcome classification and the incidents they represent, may suggest an epidemiological association between potential exposures and observed clinical effects.

Thus, given the points above, it is important not to over interpret the data and draw firm conclusions regarding cause and effect relationships from these types of spontaneously reported events alone. Clinical trials, case series (multiple cases considered together), basic animal and laboratory research and other scientific studies must test and confirm results from observational studies and hypotheses derived from epidemiological surveys before cause and effect relationships can be proposed or determined.

4.2. General results

The above limitations notwithstanding, the data does provide important insight into the safety of PY/PBO products. Of the symptomatic cases with outcomes showing effects potentially associated with exposure, approximately 87% were either asymptomatic or reported symptoms of minor severity. About 4% of the cases reported symptoms that were judged to be moderate and 0.1% of the cases reported symptoms that were severe enough to be judged as major. Less than 1% of the individuals were admitted for treatment (critical or non critical care, or psychiatric care) (data not shown).

4.3. Sensitive populations

A notable strength of the dataset relates to the depiction of a "relative" safety profile. Since calls to poison control centers typically involve both intended and unintended exposures, examination of the relative incidence of those events that are coded as adverse reaction (adverse effects following normal product use) can be quite useful in better understanding the likelihood that exposures occurring during routine product use might result in adverse effects. This would be especially useful when the incidence of a given condition (such as asthma or dust mite allergy) in the general population is known and suspected to contribute to certain injury patterns. In the case of PY/PBO products, ragweed sensitivity has often been cited as a predisposing factor to reactions from PY. Given that the incidence of ragweed sensitivity in the US is approximately 26% (Arbres et al., 2005); we would expect a high percentage of callers (approximately equal to or greater than 26%) to be reporting serious (moderate and major) symptoms for dermal or respiratory reactions if ragweed sensitivity was in fact a significant predisposing factor.

Although it is difficult to examine individual cases for specific outcomes and clinical effects and link them directly to ragweed or other allergies, it is possible to make important inferences from the data available. Insight can come from the treatment recommended to the caller by the poison control center. This sheds light on whether pyrethrins/PBO products are disproportionately responsible for reports of dermal or respiratory reactions in people with allergies (such as the often-mentioned ragweed allergy) or asthma.

Serious dermal reactions, such as those expected from allergic contact dermatitis or contact urticaria would be expected to be treated with prescription steroid use. Although OTC (Over-the-Counter) topical steroid use would more likely be associated with minor effects, the dataset does not delineate between OTC versus prescription steroid use. Still, any use of a steroid would be an indication of a dermal effect requiring some form of treatment. Thus, the use of steroids in response to a report of an adverse dermal effect is being viewed as suggestive of a more pronounced, or possibly allergic or urticarial reaction.

Similarly we can learn more about the respiratory reports by looking closely at the treatments given to those patients with respiratory responses of moderate and major severity. Use of a bronchodilator would be suggestive that an asthmatic reaction *could* be involved.

Many of the precautions about use of PY over the years have been related to belief that there was cross reactivity with people sensitive to ragweed and as well as potentially enhanced responses in people with respiratory sensitivity such as asthma. It is important to put these concerns into the context of the prevalence of ragweed sensitivity and asthma in the population.

An indication of the prevalence of allergic sensitization in the United States comes from the National Center for Health Statistics who conducted allergy skin testing in the second and third National Health and Nutritional Examination Surveys (NHANES II and III). The skin prick-puncture allergy tests for the NHANES III tested ten common allergens and two controls (positive and negative). Of the 31,311 individuals (aged 2 months to 90 years) who were interviewed and examined in the NHANES III, allergy tests were administered to all the subjects aged 6–19 years and a random half-sample of subjects aged 20–59 years. This resulted in 12,106 eligible subjects, of which 10,508 were tested and analyzed using a wheal-based definition of a positive response. Over half of the individuals tested had a positive test response to one or more allergens. Of the positive responses among the US population between 6 and 50 years of age, the most prevalent responses were to dust mite, rye, ragweed, and cockroach. The prevalence of positive test responses to short ragweed was 26.2% ± 1.03 (Arbres et al., 2005).

With respect to asthma, the CDC's (Centers for Disease Control) National Center of Health Statistics annually conducts National Health Interview Surveys (NHIS), which include questions related to asthma. These surveys provide national data on self-reported asthma prevalence, school and work days lost because of asthma, and asthma associated activity limitations (data were collected from 1980 to 1996). Additional data on self-reported asthma episodes were also collected from 1997 to 1999, along with data regarding other asthma related events or limitations. As of 1997, the NHIS began randomly collecting information regarding lifetime asthma prevalence, which includes individuals who at anytime in their lives had a medical diagnosis of asthma, and 12 month attack prevalence, which includes those with asthma who have had $\geqslant 1$ attacks or episodes in the past twelve months. In 1997, 26.7 million persons (96.6/1000 population) reported having a physician diagnosis of asthma. Only 11.1 million asthmatic individuals (40.7/1000 population) reported having an asthma attack or episode in the preceding twelve months (Mannino et al., 2002).

We have no reason to believe that the population of "sensitive" individuals would not be represented in those calling poison centers and reporting unintended or intended exposure to PY/PBO products. This is especially true as exposures often occur to inno-

cent bystanders. If the suspected population of "sensitive" individuals were, indeed sensitive to PY/PBO we would expect a much higher number of adverse effect reports and many more cases in which treatment with steroids or bronchodilators occurred. The data reviewed here do not suggest that these populations are unusually sensitive to PY/PBO.

4.4. Relative risk

Consider the relative vs. absolute risk of adverse effects reported and potentially associated with product exposure. Comparing relative risk of one symptom vs. another within a given population of all those reporting an exposure is quite different from examining the absolute risk of anyone using or being exposed to the product reporting an effect. When reviewing TESS data, there may be a tendency to focus on comparing and contrasting various clinical features of reported events within the population of those reporting without considering the relative likelihood of product use to result in a reportable incident. This is typically due to the fact that usage or "denominator" data is unavailable for most product exposures. However, if we assume, as discussed above, that over 300,000,000 people were likely to have had some exposure (either during or after product use) to PY/PBO products over the 3year study period, this would suggest that the likelihood of an individual needing to call a public poison control center for information, assessment, or treatment advice is approximately 1 in 20,000 exposures. The likelihood of an exposed individual reporting either a respiratory or dermal effect *of any outcome severity* is approximately 1 in 156,000 or 1 in 165,000 exposures, respectively.[2]

5. Conclusions

The strengths and limitations of using and interpreting TESS data must be kept in mind when drawing conclusions from these data. In spite of the limitations, the following observations were made:

- Although PY/PBO products were used extensively during the study period, serious adverse effects were rare (717 moderate and 23 major severity cases our of 17,873 calls);
- When considering adverse dermal or respiratory reactions following label-directed use of the product, adverse reactions were very rare (dermal – 17 moderate, 1 major; respiratory – 18 moderate, 0 major);
- When examining the relative frequency of either respiratory or dermal effects being reported to AAPCC after product use, the likelihood of either of those adverse symptoms being reported is approximately 1 in 1,56,000 or 1 in 1,65,000 applications, respectively, despite the overall incidence of ragweed allergy in the general population being approximately 26.2% and incidence of asthma being approximately 9.6%;
- Using bronchodilator and steroid treatment as an indication of severity of respiratory and dermal responses, respectively, the data do not suggest that asthmatics and people sensitive to ragweed are unusually sensitive to PY/PBO.

Taken as a whole and put in the context of their widespread use the data suggests that PY/PBO products can be used with a relatively low risk of adverse effects in both in people with or without asthma or allergies.

[2] The numerator comes from the number of cases reporting any dermal or respiratory symptom (Table 8).

Conflict of interest statement

Thomas G. Osimitz: Occasional consultant on toxicology issues to the Pyrethrins Joint Venture and the Piperonyl Butoxide Task Force II (PBTFII).

Nancy Sommers: There is no known potential competing interest

Richard Kingston: Employment with medical practice that contracts with pesticide manufactures to provide product related post-market medical surveillance in order for them to meet their regulatory adverse event reporting requirements.

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Appendix A

TESS. outcome severity coding definitions

- *No effect*: No signs or symptoms resulted from the exposure;
- *Minor effect*: The patient developed some signs or symptoms as a result of the exposure, but they were minimally bothersome and generally resolved rapidly with no residual disability or disfigurement. Examples of minor effects include respiratory symptoms such as coughing or respiratory irritation which resolved spontaneously with fresh air and supportive care, or dermal irritation or rash which resolved spontaneously or with minimal intervention and removal of the suspected agent from the skin;
- *Moderate effect*: The patient exhibited signs or symptoms as a result of the exposure that were more pronounced, more prolonged, or more systemic in nature than minor symptoms. Usually some form of treatment is indicated. The symptoms are not life threatening and resulted in no disfigurement or disability. An example of a moderate effect includes respiratory symptoms that required treatment with a bronchodilator or skin reactions that were more pronounced, persistent or required systemic steroid administration before resolution. Note: oral versus topical steroid use is not recorded in the dataset.
- *Major effect*: The patient exhibited signs or symptoms as a result of the exposure that were life threatening or resulted in significant residual disability or disfigurement. An example of a major respiratory effect would include respiratory arrest secondary to allergic or direct toxic reaction. Few dermal symptoms would fall into this category except for clinical effects such as full thickness, 3rd degree burns. It is unlikely that dermal symptoms alone that could be associated with PY/PBO exposure would be represented in this severity code designation.
- *Unrelated effect*: It is determined the exposure was not responsible for the reported symptoms. As an example, if a patient called the poison center suggesting that a brief incidental exposure to a product occurred on a given day and the patient was advised two weeks later that they have cancer with associated signs and symptoms, the specialist would likely code the effects as unrelated to the exposure.

Appendix B

TESS. reason for exposure

- *Adverse reaction:* An adverse event occurring with normal, prescribed, labeled or recommended use of the product, as opposed to overdose, misuse or abuse. Included are cases with an

unwanted effect due to an allergic, hypersensitive, or idiosyncratic response to the active ingredients, inactive ingredients, or excipients. Concomitant use of a contraindicated medication or food is excluded, and coded instead as a therapeutic error.

- *Intentional misuse*: An exposure resulting from the intentional improper or incorrect use of a substance for reasons *other* than the pursuit of a psychotropic effect.
- *Intentional abuse*: An exposure resulting from the intentional improper or incorrect use of a substance where the victim was likely attempting to achieve a euphoric or psychotropic effect. All recreational use of substances for any effect is included.
- *Intentional unknown*: An exposure that is determined to be intentional but the specific motive is unknown.
- *Unintentional general*: All unintentional exposures not specifically defined below. Most unintentional exposures in children are captured here. Note: This category captures any unintended exposure and is not intended to capture exposures where routine product use potentially results in adverse consequences.
- *Unintentional misuse*: Unintentional improper or incorrect use of a non-pharmaceutical substance. Unintentional misuse differs from intentional misuse in that the exposure was unplanned or not foreseen by the patient.
- *Unintentional unknown*: An exposure determined to be unintentional, but the exact reason is unknown.

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A Human-Health Risk Assessment for West Nile Virus and Insecticides Used in Mosquito Management

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West Nile virus (WNV) has been a major public health concern in North America since 1999, when the first outbreak in the Western Hemisphere occurred in New York City. As a result of this ongoing disease outbreak, management of mosquitoes that vector WNV throughout the United States and Canada has necessitated using insecticides in areas where they traditionally have not been used or have been used less frequently. This has resulted in concerns by the public about the risks from insecticide use. The objective of this study was to use reasonable worst-case risk assessment methodologies to evaluate human-health risks for WNV and the insecticides most commonly used to control adult mosquitoes. We evaluated documented health effects from WNV infection and determined potential population risks based on reported frequencies. We determined potential acute (1-day) and subchronic (90-day) multiroute residential exposures from each insecticide for several human subgroups during a WNV disease outbreak scenario. We then compared potential insecticide exposures to toxicologic and regulatory effect levels. Risk quotients (RQs, the ratio of exposure to toxicologic effect) were < 1.0 for all subgroups. Acute RQs ranged from 0.0004 to 0.4726, and subchronic RQs ranged from 0.00014 to 0.2074. Results from our risk assessment and the current weight of scientific evidence indicate that human-health risks from residential exposure to mosquito insecticides are low and are not likely to exceed levels of concern. Further, our results indicate that, based on human-health criteria, the risks from WNV exceed the risks from exposure to mosquito insecticides.
Key words: **comparative risk assessment, mosquito control, organophosphates, pesticide exposure, pyrethroids, risk analysis, vectorborne disease.** ***Environ Health Perspect*** **114:366–372 (2006). doi:10.1289/ehp.8667 available via *http://dx.doi.org/* [Online 28 October 2005]**

West Nile virus (WNV) has become a major public health concern in North America since 1999, when the first outbreak in the Western Hemisphere occurred in New York City, causing 62 cases of human encephalitis and 7 deaths [Centers for Disease Control and Prevention (CDC) 1999]. The initial outbreak in New York City is thought to have affected 2.6% of the population (Hubalek 2001). In 2000, WNV spread to three states, with 21 human cases of WNV infection and 2 deaths. In 2001, 66 human cases and 9 deaths were reported in 10 states, before WNV spread westward, affecting all but 6 states in 2002 and causing the largest arboviral encephalitis epidemic in U.S. history (Huhn et al. 2003). A total of 4,156 human cases were documented, with 284 deaths reported (CDC 2003), and numbers continued to grow in 2003, when 46 states reported 9,862 human cases with 264 deaths (CDC 2004a). In 2004, 2,539 human cases and 100 deaths were reported in 41 states (Hayes et al. 2005). Since the first appearance of WNV in the United States in 1999, the CDC has reported 16,706 documented human cases and 666 deaths (CDC 2004b; Hayes et al. 2005); however, large numbers of human infections may not be detected because of significant underreporting of milder cases of West Nile fever (Hubalek 2001; Huhn et al. 2003). Given the infection rate observed for previous years, Peleman (2004) estimated that 1.5 million people were infected with the virus in 2003.

As a result of this ongoing disease outbreak, management of mosquitoes that vector WNV throughout the United States and Canada has necessitated using insecticides in areas where they traditionally have not been used or have been used less frequently. This practice has raised concerns by the public about risks from insecticide use. In a survey by Hinten (2000), 54% of 880 people surveyed were either equally afraid of WNV and pesticides or were more afraid of the insecticides. Since 1999, numerous concerns have been raised by the public regarding the safety of using insecticides to control mosquitoes (Cohen 2003; Fehr-Snyder 2004; Fitz 2003). Some of those concerned have even suggested that the health risks from the insecticides exceed those of WNV (Cohen 2003; Ziem 2005). These concerns by the public are not exclusive to the WNV issue, but reflect long-standing perceptions of risk from pesticides (Peterson and Higley 1993; Slovic 1987).

Risk assessment is a formalized basis for the objective evaluation of risk in which assumptions and uncertainties are clearly considered and presented [National Research Council (NRC) 1983, 1996]. Human-health and ecologic risk can be described in quantitative terms as a function of effect (also termed "hazard" or "toxicity") and exposure (NRC 1983). Risk assessment typically uses a tiered modeling approach extending from deterministic models (tier 1) based on conservative assumptions to probabilistic models (tier 4) using refined assumptions [Society for Environmental Toxicology and Chemistry (SETAC) 1994]. In risk assessment, conservative assumptions in lower-tier assessments represent overestimates of effect and exposure; therefore, the resulting quantitative risk values typically are conservative and err on the side of safety.

Unfortunately, few, if any, science-based considerations of the risks of insecticide use versus the risks from vectorborne diseases have been examined. An understanding of the human-health risks for both vectorborne diseases and associated vector controls would aid greatly in decision making by all stakeholders. Therefore, the objective of this study was to use risk assessment methodologies to evaluate human-health risks from WNV and from the insecticides used to control adult mosquitoes.

Materials and Methods

Problem formulation. Although WNV has important effects on horses and birds, our assessment of health risks from WNV focused only on humans. Currently, effect and exposure factors for WNV are poorly understood (Loeb et al. 2005), making quantitative modeling of risk difficult. Therefore, we evaluated documented health effects from WNV infection and determined potential population risks based on reported frequencies. Because of the relatively recent emergence of WNV in North America, information on prevalence of various effects of the disease should be regarded as tentative.

Our tier-1 quantitative assessment of human-health risks associated with insecticides

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used in mosquito control focused on acute and subchronic residential exposures after truck-mounted ultra-low-volume (ULV) spraying of mosquito adulticides. The dissemination of mosquito adulticides by ULV application generates fine aerosol droplets that remain aloft and target flying mosquitoes [U.S. Environmental Protection Agency (EPA) 2002b]. Acute exposures were defined as single-day exposures immediately after a spray event. Subchronic exposures were defined as exposures per day over a 90-day seasonal multispray event. A total of 10 spray events were assumed to occur on days 1, 4, 14, 17, 27, 30, 40, 43, 53, and 56. This design represents a reasonable worst-case mosquito insecticide seasonal application scenario, including during a human epidemic of WNV [Karpati et al. 2004; New York City Department of Health (NYCDOH) 2001]. Chronic exposures (> 6 months) to mosquito adulticides are unlikely. Additionally, extrapolation of subchronic exposures to chronic exposure time frames would result in lower risks than with subchronic risks (NYCDOH 2001). Therefore, chronic risks were not assessed in this study.

Exposures to several population subgroups were estimated to account for potential age-related differences in exposure. Groups included adult males, adult females, infants (0.5–1.5 years of age), and children (2–3, 5–6, and 10–12 years of age). Adult males were assumed to weigh 71.8 kg, which represents the mean body weight for all males > 18 years of age, and adult reproductive females were assumed to weigh 60 kg, which represents the mean body weight for females between 13 and 54 years of age (U.S. EPA 1996). Children 5–6 and 10–12 years of age were assumed to weigh 21.1 and 40.9 kg, respectively. Infants (0.5–1.5 years of age) and toddlers (2–3 years of age) were assumed to weigh 9.4 and 14.3 kg, respectively. All weights for children were derived from mean body weight values for male and female children within their respective age groups (U.S. EPA 1996).

Hazard identification. We conducted human-health risk assessments for six insecticide active ingredients (permethrin, pyrethrins, resmethrin, phenothrin, malathion, and naled) and one synergist (piperonyl butoxide). Malathion and naled are in the organophosphate class of insecticides, and permethrin, pyrethrins, resmethrin, and phenothrin are in the pyrethroid class. The synergist, piperonyl butoxide, is present in many formulations with pyrethroids. All compounds are currently registered by the U.S. EPA for adult mosquito management in the United States.

Toxicity end points. Toxicity and dose–response information for each compound were reviewed for acute and subchronic exposure durations. Toxicity end points in this assessment were chosen based on U.S. EPA regulatory end points.We used inhalation, dermal, and ingestion toxicity end points for each respective exposure route and duration. Ingestion reference doses (RfDs) were used as the toxicity end points (acceptable daily exposures) and were compared with total estimated exposures (total body burden). Acute and subchronic ingestion RfDs were calculated by dividing the most sensitive toxic effect [typically the no observed adverse effect level (NOAEL)] by a series of uncertainty factors (typically a factor of 100 to account for intraspecies and interspecies uncertainty) (Table 1).

Environmental concentrations and fate of insecticides. We used the AERMOD, version 1.0 tier 1 air dispersion model (U.S. EPA 1999) to predict the 7.6 m (25 ft) and 91.4 m (300 ft) air concentrations (micrograms per cubic meter) of each insecticide within 1- and 6-hr time ranges after ULV application by a truck-mounted sprayer. Estimates of environmental concentrations are presented only for truck-mounted ULV applications because our modeling suggested that delivery of ULV applications by aircraft resulted in substantially less aerial and surface deposition (and therefore less human exposure and risk). This was also observed by the NYCDOH (2001).

We used the following conservative assumptions: *a*) each chemical had a 24-hr half-life in air except for naled, which was given a 18-hr half-life; *b*) the insecticides were applied at the maximum application rate as stated on each label; *c*) all of the insecticides were susceptible to the same weather conditions using standardized weather data from Albany, New York, in 1988; *d*) all spray events occurred at 2100 hr; and *e*) each spray release was at 1.5 m. The chemical properties, application rates, and predicted environmental concentrations for each active ingredient are listed in Table 2.

Receptors were established within the model on a Cartesian grid at five intervals of

Table 1. Toxicologic effects and regulatory end points for the active ingredients.

Compound	Acute		Subchronic	
	End point	Study and toxicologic effects	End point	Study and toxicologic effects
Malathion	NOAEL = 50 mg/kg/day[a] RfD = 0.5 mg/kg/day UF = 100	Based on reduction in maternal bw gain in a study with pregnant rabbits[a]	NOAEL = 2.4 mg/kg/day[a] RfD = 0.024 mg/kg/day UF = 100	Based on inhibition of blood enzyme activity at 50 ppm malathion in the diet in a 24-month study in rats[a]
Naled	NOAEL = 1.0 mg/kg/day[b] RfD = 0.01 mg/kg/day UF = 100	Based on inhibition of blood and brain enzymes in a 28-day study in rats[b]	NOAEL = 1.0 mg/kg/day[b] RfD = 0.01 mg/kg/day UF = 100	Based on inhibition of blood and brain enzymes in a 28-day study in rats[b]
Permethrin	NOAEL = 25 mg/kg/day[c] RfD = 0.25 mg/kg/day UF = 100	Acute neurotoxicity study in rats LOEL = 75 mg/kg based on observations of clinical signs such as aggression, abnormal/decreased movement, and increased body temperature[c]	NOAEL = 25 mg/kg/day[c] RfD = 0.25 mg/kg/day UF = 100	Acute neurotoxicity study in rats LOEL = 75 mg/kg based on observations of clinical signs such as aggression, abnormal/decreased movement, and increased body temperature[c]
Resmethrin	NOEL = 10 mg/kg/day[d] RfD = 0.1 mg/kg/day UF = 100	Based on liver weight increases in a 6-month study in dogs[d]	NOEL = 10 mg/kg/day[d] RfD = 0.1 mg/kg/day UF = 100	Based on liver weight increases in a 6-month study in dogs[d]
Phenothrin	NOEL = 70 mg/kg/day[e] RfD = 0.7 mg/kg/day UF = 100	13-week study in rats LOEL = 216 mg/kg-day based on increases in liver weights and decreases in cholesterol in both male and female rats[e]	NOEL = 70 mg/kg/day[e] RfD = 0.7 mg/kg/day UF = 100	13-week study in rats LOEL = 216 mg/kg-day based on increases in liver weights and decreases in cholesterol in both male and female rats[e]
Pyrethrins	NOAEL = 20 mg/kg/day[f] RfD = 0.07 mg/kg/day UF = 300	Acute neurotoxicity study in rats LOAEL = 63 mg/kg/day based on tremors in females[f]	NOAEL = 4.37 mg/kg/day[f] RfD = 0.044 mg/kg/day UF = 100	Rat chronic toxicity study LOAEL = 42.9 mg/kg/day based on increased incidence of thyroid follicular cell hyperplasia in males.[f]
Piperonyl butoxide	NOAEL = 630 mg/kg/day[g] RfD = 6.3 mg/kg/day UF = 100	Developmental toxicity study in rats LOAEL = 1,065 mg/kg/day based on decreases in maternal bw gain[g]	NOAEL = 89 mg/kg/day[g] RfD = 0.89 mg/kg/day UF = 100	Two generation reproduction study in rats LOAEL = 469 mg/kg/day based on decrease in bw gain of F_1 and F_2 pups at postnatal day 2[g]

Abbreviations: bw, body weight; LOAEL, lowest observed adverse effect level. LOEL, lowest observed effect level; NOEL, no observed effect level; UF, uncertainty factor used to determine the RfD.
[a]U.S. EPA 2000c. [b]U.S. EPA 2002a. [c]U.S. EPA 2005c. [d]U.S. EPA 2000a. [e]U.S. EPA 2000b. [f]U.S. EPA 2005b. [g]U.S. EPA 2005a.

25 m at 7.6 m and 91.4 m from the edge of the spray emission area. The receptors were at a height of 1.5 m. Each receptor estimated the 1- and 6-hr average air concentrations for each insecticide. An average was then taken of the estimates from the six receptors at 7.6 m that were not at the edges of the spray zone. The following data were obtained using this network of receptors: the 1-hr average concentration at 7.6 m, the 6-hr average at 7.6 m, and the peak value at 91.4 m.

We used the screening Industrial Source Complex Short-Term (ISCST3) model (U.S. EPA 1995) to estimate particle deposition (milligrams per square meter) at 7.6 m and 91.4 m from the spray area at a 1-hr average. The following assumptions were made in addition to those from AERMOD: *a*) all of the insecticides were susceptible to the same weather conditions using standardized weather data from Salem, Massachusetts; *b*) the ULV particle size applications had 3% of the emitted particles greater than the allowable particle size as stated on the label; and *c*) the particles were assigned a density in accordance with the specific gravity of each insecticide.

A Cartesian grid was used for ISCST3 that was similar to that used in AERMOD. Receptors were added at 15.24-m intervals between 7.6 m and 91.4 m from the spray source to obtain a more accurate estimate of the average deposition within 91.4 m of the source. The receptors were also at the same height of 1.5 m. All of the same methods were used to calculate the average deposition at 7.6 m and 91.4 m. The middle receptors were included to calculate an average deposition within 91.4 m. The following data were obtained from this information: deposition at 7.6 m, deposition at 91.4 m, and the average deposition within 91.4 m of the spray source.

For estimating subchronic exposures, we used the estimated deposition values within 91.4 m for each insecticide in an exponential decay model to characterize their persistence on surfaces such as soil within a spray program that included 10 sprays on days 1, 4, 14, 17, 27, 30, 40, 43, 53, and 56. Insecticide concentrations for each spray event were followed through day 90 using the following multiple degradation model:

$$D = \sum_{j=i}^{90} P e^{(r_1+r_2)t}, \qquad [1]$$

where D is the sum of the deposition over one spray, P is the peak deposition after a spray event, r_1 is the rate of decay calculated by using the aerobic soil half-life of each active ingredient, r_2 is the rate of decay calculated by using the soil photolysis half-life of each active ingredient, t is the time in hours, and j is the spray day. The average daily exposure was then determined by dividing the deposition sum by 90.

The same deposition and degradation model was used to characterize deposition and persistence on garden produce by using a Kenaga nomogram to estimate the deposition (milligrams per kilogram dry weight) of each insecticide on respective plant parts. Because the nomogram represents a linear relationship between application rate and maximum residues, it can be used to estimate the maximum residues on plant surfaces for a given application rate (Fletcher et al. 1994). For this analysis, maximum application rates were used for each insecticide, and each estimated concentration was then applied to the model above, using the surface photolysis half-life to estimate the rate of degradation.

Acute exposure. We assumed that multiroute exposures immediately after a single-spray event were limited to 24 hr. Routes of insecticide exposure included inhalation, dermal contact with spray, hand-to-mouth ingestion by infants and toddlers from spray deposition on hands, and ingestion of garden produce. We also assumed that residents did nothing to limit their exposure to the spray. In its assessment of acute and subchronic exposures from several mosquito adulticides, the NYCDOH (2001) concluded that exposures from potable water and swimming were negligible. Using environmental fate models, we also concluded that the chemical properties of the insecticides result in negligible concentrations in water. Therefore, we did not include these exposures in our assessment.

Acute inhalation exposure. Acute inhalation exposures were estimated as

$$PE = (EEC \times RR \times D \times CF) \div bw, \qquad [2]$$

where PE is potential exposure [milligrams per kilogram body weight (bw)], EEC is the 6-hr average estimated environmental concentration of an active ingredient in the air 1.5 m high at 7.6 m from the spray source (micrograms per cubic meter), RR is the respiratory rate under moderate activity (cubic meters per hour), D is the duration of exposure (hours), CF is the conversion factor to account for the conversion of units from micrograms per cubic meter to milligrams per cubic meter, and bw is body weight (kilograms).

RRs were assumed to be 1.6 m^3/hr for adults and 1.2 m^3/hr for children, including infants. These rates are indicative of moderate physical activity (U.S. EPA 1996). The duration of exposure was 6 hr. Therefore, the assumption was that the person was outside and 7.6 m from the spray truck when it passed him or her. Moreover, the person remained outside, 7.6 m from the emission, for the following 6 hr, respiring as if under moderate physical activity during the entire time. Body weight for the different age groups is discussed above.

Acute dermal exposure from spray deposition. Acute dermal exposures from deposition of spray drift on skin were estimated as

$$PE = (TDE \times AB) \div bw, \qquad [3]$$

where PE is potential exposure (milligrams per kilogram bw), TDE is total dermal exposure (milligrams), AB is dermal absorption rate, and bw is body weight (kilograms). There is no publicly available information on dermal deposition immediately after truck-mounted ULV sprays. Therefore, we used the U.S. EPA Pesticide Handler Exposure Database (PHED; U.S. EPA 1998) as a conservative surrogate. The PHED contains pesticide-handler scenarios derived from field studies and exposure estimates based on physical factors such as application rate, hectares treated per day, type of clothing worn, methods of application, and

Table 2. Application rates, chemical properties, and predicted environmental concentrations of active ingredients.

Property	Active ingredient						
	Piperonyl butoxide	Phenothrin	Permethrin	Resmethrin	Malathion	Naled	Pyrethrins
Application rate (kg ai/ha)	0.0392	0.004	0.0078	0.0078	0.0639	0.0224	0.009
Density (g/mL)	0.898[a]	0.898[a]	0.8657[b]	0.87[c]	1.23[d]	1.67[e]	0.81[f]
Surface photolysis half-life (days)	NA[g]	6[c]	23[h]	0.14[i]	6.5[i]	2.4[i]	0.5[j]
Soil aerobic half-life (days)	14[i]	7[i]	37[k]	30[h]	1[h]	1[h]	1[j]
Acute air concentration (μg/m^3)[l]	7.39	0.81	1.55	1.61	9.76	1.68	1.7
1-Day acute produce concentration (mg/kg dry wt)	0.525	0.054	0.105	0.105	0.855	0.3	0.12
90-Day mean surface concentration (mg/m^2)[m]	15.42	0.43	4.14	0.22	2.18	0.65	0.54
90-Day mean produce concentration (mg/kg dry wt)	2.88	0.055	0.096	0.012	0.73	0.13	0.21

Abbreviations: ai/ha, active ingredient per hectare; NA, not available; wt, weight.
[a]Clarke Mosquito Control Products (1999b). [b]Clarke Mosquito Control Products (1999a). [c]Bayer Environmental Science (2004). [d]Griffin (2001). [e]AMVAC (2003). [f]McLaughlin Gormley King Co. (2004). [g]Surface and produce concentrations determined from soil aerobic half-life only. [h]U.S. Department of Agriculture (USDA 2005). [i]NYCDOH (2001). [j]Food and Agricultural Organization (2000). [k]U.S. EPA (2005c). [l]6-Hr mean concentration at 7.6 m from spray source. [m]90-Day mean surface concentration within 91.4 m of the spray source.

formulation type. We used the PHED scenario in which a flagger (person marking the location for pesticide application while the application is occurring) was exposed to a liquid application. We assumed that the person was not wearing clothing and that the exposure was 10 times greater than the flagger scenario. We believe this scenario conservatively estimated residential dermal exposure for two reasons: *a*) we added a 10-fold increase in exposure, and *b*) the U.S. EPA has not considered acute dermal contact from ULV applications for pyrethrins, piperonyl butoxide, and permethrin because it was believed to be negligible (U.S. EPA 2005a, 2005b, 2005c). The values for percent dermal absorption were 0.22% for pyrethrins (U.S. EPA 2005b), 2% for piperonyl butoxide (U.S. EPA 2005a), 10% for malathion and resmethrin (U.S. EPA 2000a, 2000c), 15% for permethrin (U.S. EPA 2005c), 70% for phenothrin (U.S. EPA 2000b), and 100% for naled (U.S. EPA 2002a).

Acute hand-to-mouth exposure from spray deposition on hands. Acute hand-to-mouth exposures were estimated for only two subgroups (toddlers and infants), because young children are more likely than adults to be exposed to pesticides as a result of hand-to-mouth contact (Cohen Hubal et al. 2000). Exposures were calculated as

$$PE = [(THD \div HSA) \times AHS \times SEF] \div bw, \quad [4]$$

where *PE* is potential exposure (milligrams per kilogram bw), *THD* is total hand dermal exposure (milligrams), *HSA* is adult hand surface area (square meters), *AHS* is adjusted hand surface area for each subgroup (square meters), *SEF* is saliva extraction factor, and *bw* is body weight (kilograms). Total hand dermal exposure was determined using the PHED database and the assumptions discussed above. The hand surface area of toddlers (2–3 years of age) was assumed to be 0.035 m^2, which represents the 50th percentile total surface area values for males and females in the 2–3 and 3–4 year age groups, multiplied by the mean percentage of the total body represented by hands for males and females of that age (U.S. EPA 1996). The hand surface area for infants was assumed to be 0.007 m^2 and was also calculated as a percent of total body surface area for infants (U.S. EPA 1996). We calculated the total body surface area of infants using the formula by Current (1998). We assumed that, on the day of application, 50% of the insecticide deposited on the hand was available through saliva extraction (U.S. EPA 2005a, 2005c).

Acute ingestion of garden produce. We assumed that the insecticide settled onto a tomato garden and that the resident picked, processed, and ate tomatoes the next day. The estimated maximum insecticide residue deposited on tomatoes is discussed above. We assumed that the resident did not wash the tomatoes after picking. The residue concentration also did not change with processing of the tomatoes. The amount of insecticide ingested was estimated as the product of the residue concentration and the quantity of food consumed. Tomato consumption patterns were determined using the Dietary Exposure Evaluation Model [(DEEM)-Food Commodity Intake Database (FCID) version 2.04; Exponent, Washington, DC]. The model determines dietary consumption for the U.S. population and several subgroups by using individual food consumption records collected by the U.S. Department of Agriculture (USDA) Continuing Surveys for Food Intake by Individuals for 1994–1998. Translation factors used to convert foods-as-eaten to commodities are based on a U.S. EPA/USDA FCID recipe set. For this assessment, we determined the acute food consumption patterns by subgroup using the 95th percentile 1-day consumption values for tomatoes, tomato baby food, tomato paste, tomato paste baby food, tomato puree, tomato puree baby food, dried tomato, dried tomato baby food, and tomato juice. Therefore, the respective individuals in these subgroups ate all of these tomato food products within 1 day of application at the 95th percentile of U.S. national consumption.

Subchronic exposure. We assumed multiroute exposures per day over 90 days after multispray events. Routes of insecticide exposure included inhalation, dermal contact with spray, ingestion of garden produce, hand-to-mouth ingestion by infants and toddlers from spray deposition on hands, hand-to-mouth ingestion by infants and toddlers from deposition on surfaces, dermal contact with soil and other surfaces, and soil ingestion.

Subchronic inhalation, dermal, and hand-to-mouth exposures. Exposures for each exposure type were estimated as

$$PE = (PE_{acute,\ type} \times SE) \div D, \quad [5]$$

where *PE* is the potential exposure (milligrams per kilogram bw per day), $PE_{acute,\ type}$ is the acute exposure type (e.g., acute inhalation) from each spray event (milligrams per kilogram bw), *SE* is the number of spray events, and *D* is the duration of exposure (days). We assumed that the insecticides were sprayed on days 1, 4, 14, 17, 27, 30, 40, 43, 53, and 56 (10 spray events per season) in any given area. The exposure duration was 90 days.

Subchronic hand-to-mouth exposure from deposition on surfaces. Subchronic hand-to-mouth exposures were estimated for only two subgroups (toddlers and infants) based on the rationale discussed above. Exposures were calculated as

$$PE = (EEC \times SEF \times SA \times DR \times FA \times D) \div bw, \quad [6]$$

where *PE* is potential exposure (milligrams per kilogram bw per day), *EEC* is the 90-day average environmental concentration of the active ingredient deposited on soil or turf within 91.4 m from the spray source (milligrams per square meter), *SEF* is saliva extraction factor, *SA* is surface area for three fingers (square meters), *DR* is dislodgeable residue, *FA* is frequency of activity (events per hour), *D* is exposure duration (hours), and *bw* is body weight (kilograms). Assumptions for estimating subchronic environmental concentrations are discussed above. The saliva extraction factor was assumed to be 50% (U.S. EPA 2005a, 2005c), and the palmar surface area for three fingers was assumed to be 20 cm^2 (U.S. EPA 2005c). Dislodgeable insecticide residue from soil or turf grass was assumed to be 20% (U.S. EPA 1997). The frequency of hand-to-mouth activity in children was assumed to be 20.5 events/hr and is based on the maximum frequency observed (Freeman et al. 2005). The duration of exposure was assumed to be 4 hr/day. Therefore, the toddler or infant was assumed to be engaging in hand-to-mouth activities outside each day for 4 hr over 90 days.

Subchronic ingestion of garden produce. Our assumptions for subchronic ingestion of garden produce were the same as for acute ingestion of produce, with the following differences: *a*) the insecticide was deposited onto both tomatoes and head- and leaf-lettuce, *b*) all tomato and lettuce consumption by the residents over the 90 days was from the garden, and *c*) tomato and lettuce consumption patterns were determined using chronic food consumption patterns (3-day average).

Subchronic dermal contact with soil and other surfaces. Exposures from contact with soil, turf, and other outdoor surfaces were calculated as

$$PE = (EEC \times SA \times SS \times AB \times DR \times CF) \div bw, \quad [7]$$

where *PE* is potential exposure (milligrams per kilogram bw per day), *EEC* is the 90-day average environmental concentration of the active ingredient deposited on soil or turf within 91.4 m from the spray source (milligrams per square meter), *SA* is body surface area in contact with surface (square centimeters), *SS* is weight of soil adhered to skin (milligrams per square centimeter), *AB* is dermal absorption rate, *DR* is dislodgeable residue, *CF* is the conversion factor to account for square meters to square centimeters, and *bw* is body weight (kilograms). The body surface area in contact with the surface was assumed to be the sum of surface areas for face (head ÷ 2), hands, arms, legs, and feet (U.S. EPA 1996). Therefore, we

assumed residents were minimally clothed while outside. Contact with surfaces was associated with certain human activities. The activities were assumed to be gardening for adults (0.55 mg soil/cm^2 skin) and soccer for children, including infants (0.164 mg soil/cm^2 skin) (U.S. EPA 1996). We assumed that these activities occurred each day over the 90 days. The assumptions for dermal absorption rate and dislodgeable residues are discussed above.

Subchronic soil ingestion. Exposures from incidental ingestion of soil were calculated as

$$PE = [(EEC \div SW) \times SI] \div bw, \qquad [8]$$

where *PE* is potential exposure (milligrams per kilogram bw per day), *EEC* is the 90-day average environmental concentration of the active ingredient deposited on soil or turf within 91.4 m from the spray source (milligrams per square meter), *SW* is soil weight (milligrams per cubic meter), *SI* is soil ingestion (milligrams per day), and *bw* is body weight (kilograms). Because the insecticide would only be surface-deposited on soil, we assumed that the concentration (milligrams per square meter) would be the same for a cubic meter of soil. Soil weight was assumed to be 3.86 kg/m^3 based on reported densities for Scotts lawn soil (The Scotts Company, Marysville, OH). Soil ingestion rates were assumed to be 100 mg/day for children and 50 mg/day for adults (U.S. EPA 1996). We assumed that all soil ingestion each day was from soil containing residues of the active ingredients.

Risk characterization. Human-health risks in this study were assessed by integrating toxicity and exposure. We assessed risks using the risk quotient (RQ) method. For each population subgroup, an RQ was calculated by dividing the PE by the appropriate toxicity end point (e.g., the RfD). Therefore, the RQ is the ratio of exposure to effect. RQs < 1 are typically below regulatory levels of concern.

Exposures by similar route of exposure and duration (e.g., subchronic dermal contact with spray and surfaces) were compared with the appropriate RfD (e.g., subchronic dermal RfD). Multiroute exposures (dermal + ingestion + inhalation) were compared with the ingestion RfD. The ingestion RfD provided a conservative toxicity end point because it typically was based on the most sensitive NOAEL. Therefore, it represented the largest dose in which no adverse effects on human health would occur during the relevant exposure duration.

Results

West Nile virus risks. According to a seroepidemiologic survey conducted by Mostashari et al. (2001), for every diagnosed case of West Nile (WN) meningoencephalitis, there were approximately 30 additional people with WN fever, and approximately 2.6% of the population in outbreak areas in New York were infected during the epidemic of 1999. Loeb et al. (2005) reported a 3.1% outbreak infection rate in Oakville, Ontario, Canada, in 2002. Unfortunately, the seroprevalence of WNV antibodies across larger time and geographic scales has not been determined. Overall, 20% of infected persons develop mild febrile illness (Mostashari et al. 2001), and 0.67% develop neurologic disease (Fratkin et al. 2004). A total of 0.43% develop encephalitis, and 0.24% develop meningitis (Asnis et al. 2001; Brilla et al. 2004; Emig and Apple 2003; Klee et al. 2004; Sejvar et al. 2003a; Weiss et al. 2001).

Case-fatality rates in the United States ranged from 4 to 18% among hospitalized patients (Brilla et al. 2004; Emig and Apple 2003; Nash et al. 2001b; Pepperell et al. 2003; Sejvar et al 2003a; Weiss et al. 2001) and from 2.7 to 14% among cases reported to the CDC (CDC 2004b).

No difference in distribution of WNV infection among age groups and between sexes is apparent (Nash et al. 2001a, 2001b; Tyler 2001), but for unknown reasons, males seem to be at higher risk for WN neuroinvasive illness (O'Leary et al. 2004; Petersen and Marfin 2002). Children infected with WNV usually show no symptoms or have only a mild fever (Hayes and O'Leary 2004). The incidence of encephalitis and death increases with age (Nash et al. 2001a, 2001b; O'Leary et al. 2004; Tsai et al. 1998; Weinberger et al. 2001). Weiss et al. (2001) reported that persons ≥ 50 years of age were more likely to present meningoencephalitis and had increased mortality rates; other reports show that the incidence of neurologic symptoms and death may increase 10- to 20-fold among persons ≥ 50 years of age (Nash et al. 2001a; Sampathkumar 2003; Tyler 2001). The risk increases 43 times for persons ≥ 80 years of age (Sampathkumar 2003).

Few data exist regarding long-term morbidity after WNV infection. Substantial morbidity may follow hospitalization for WNV infection (Petersen et al. 2003) and is observed in patients with WN fever (Watson et al. 2004). Encephalitis cases seem to have more variable outcomes than meningitis cases, which tend to recover well (Granwehr et al. 2004). A poor prognosis and very limited recovery have been observed in acute flaccid paralysis cases (Saad et al. 2005; Sejvar et al. 2003a, 2003b).

Although patients with WN fever tend to recover well, median recovery time was 60 days for patients in Illinois in 2002 (Watson et al. 2004). The disease also has a significant effect on the lifestyle of patients with WN fever. Of 98 respondents with WN fever, 57 (58%) missed work/school, 82 (84%) had household activities limited, 47 (49%) had difficulty walking, and 89 (91%) had outside-of-home activities limited (Watson et al. 2004).

In a long-term follow-up study on 42 WN encephalitis survivors 1 year after illness onset, only 37% presented full physical, functional, and cognitive recoveries, and there was a substantially higher prevalence of impairment compared with baseline (Nash et al. 2001a). Similarly, only 2 of 8 patients in a study in New York presented full recovery after 1 year; 3 patients had neurologic sequelae, and 1 patient had minimal impairment after 18 months (Asnis et al. 2001).

Acute risks from insecticides. Table 3 shows the calculated RQs for each active ingredient in terms of total acute PE. Exposures and risks also were determined for each exposure route. Potential acute inhalation exposures of the six human subgroups to the adulticides ranged from 0.00011 to 0.0075 mg/kg bw, and the environmental concentrations were lower than

Table 3. Acute RQs for the active ingredients for each subgroup.[a]

Subgroup	Malathion	Naled	Permethrin	Resmethrin	Phenothrin	Pyrethrins	Piperonyl butoxide
Adult males	0.0076	0.1496	0.0020	0.0052	0.0004	0.0081	0.0004
Adult females	0.0079	0.1576	0.0021	0.0055	0.0004	0.0085	0.0004
Children (10–12 years)	0.0105	0.2123	0.0029	0.0072	0.0006	0.0113	0.0006
Children (5–6 years)	0.0177	0.3631	0.0049	0.0123	0.0010	0.0190	0.0009
Toddlers (2–3 years)	0.0225	0.4726	0.0063	0.0159	0.0013	0.0245	0.0012
Infants (0.5–1.5 years)	0.0188	0.4495	0.0058	0.0147	0.0012	0.0218	0.0010

[a]RQ = total acute PE ÷ RfD.

Table 4. Subchronic RQs for the adulticides for each subgroup.[a]

Subgroup	Malathion	Naled	Permethrin	Resmethrin	Phenothrin	Pyrethrins	Piperonyl butoxide
Adult males	0.0360	0.0259	0.0007	0.0004	0.0001	0.0056	0.0032
Adult females	0.0363	0.0269	0.0007	0.0004	0.0001	0.0056	0.0032
Children (10–12 years)	0.0470	0.0290	0.0008	0.0005	0.0001	0.0074	0.0043
Children (5–6 years)	0.0676	0.0447	0.0012	0.0009	0.0002	0.0104	0.0059
Toddlers (2–3 years)	0.1815	0.1294	0.0204	0.0037	0.0009	0.0270	0.0262
Infants (0.5–1.5 years)	0.2074	0.1661	0.0301	0.0054	0.0013	0.0292	0.0325

[a]RQ = total subchronic PE ÷ RfD.

the inhalation reference concentrations for all active ingredients evaluated. Potential acute dermal exposures to the adulticides ranged from 0.0000001 to 0.0011 mg/kg bw, with RQs ranging from 0.0000005 to 0.0113. For acute exposure due to ingestion (hand-to-mouth exposure from spray deposition on hands and ingestion of produce), total PEs ranged from 0.0001 to 0.0061 mg/kg bw, with RQs ranging from 0.00014 to 0.2142. Total acute RQs ranged from 0.0004 to 0.4726.

Subchronic risks from insecticides. Table 4 shows the calculated RQs for each active ingredient in terms of total subchronic PE. Potential subchronic inhalation exposures of the six subgroups to the adulticides ranged from 0.000012 to 0.00083 mg/kg bw. For subchronic dermal exposures to the adulticides (dermal and contact with soil), total PEs ranged from 0.00000006 to 0.00015 mg/kg, with RQs ranging from 0.0000001 to 0.0015. Potential subchronic exposures due to ingestion (ingestion of produce and soil, hand-to-mouth activity after contact with surfaces, and hand-to-mouth activity after contact with spray drift) ranged from 0.00001 to 0.0283 mg/kg bw, with RQs ranging from 0.00007 to 0.1709. Total subchronic RQs ranged from 0.00014 to 0.2074.

None of the subgroups had RQs ≥ 1.0 (i.e., PEs did not equal or exceed the RfDs) for any of the active ingredients evaluated. The lowest acute RQs were to phenothrin and piperonyl butoxide for adults and the highest acute RQ was to naled for toddlers (Table 3). The lowest and highest subchronic RQs were to phenothrin for adults and malathion for infants, respectively (Table 4).

Discussion

Conservatism. Based on the exposure and toxicity assumptions above, we believe our assumptions were sufficiently conservative and most likely overestimated risk. For example, assuming an acute RR of 0.8 m^3/hr for 2 hr and no dermal or ingestion exposures [which were the U.S. EPA assumptions for mosquito control uses of permethrin (U.S. EPA 2005c)], there would be a 90% reduction in exposure for toddlers compared with our value. Indeed, draft tier 1 risk assessments recently conducted for malathion, piperonyl butoxide, pyrethrins, and permethrin by the U.S. EPA also suggest that our results are sufficiently conservative (U.S. EPA 2000c, 2005a, 2005b, 2005c). Because of the conservative exposure assumptions used, we believe higher-tiered risk assessments using more realistic exposures would result in risk values significantly lower than those presented here.

The conservatism of our risk assessments for insecticides used in adult mosquito control is supported by residential biomonitoring and epidemiologic studies. Currier et al. (2005) assessed human exposure to ULV-applied naled, permethrin, and phenothrin in Mississippi, North Carolina, and Virginia as a result of emergency large-scale mosquito abatement. Using biomonitoring of urine, they did not observe an increase in insecticide metabolite concentrations among exposed residents. Karpati et al. (2004) and O'Sullivan et al. (2005) did not observe increases in hospital emergency department visits for asthma after wide-scale spraying of residential neighborhoods.

Uncertainties. Despite the conservatism of our risk assessment, uncertainties were revealed. Many of the uncertainties associated with residential exposure estimates are discussed above. The principal uncertainty was for environmental concentrations of the active ingredients. Data for actual aerial concentrations and surface deposition of active ingredients need to be generated to more accurately characterize risks. Because of the nature of ULV application methods, it is likely that concentrations of active ingredients are much lower than those predicted using the AERMOD and ISCST3 tier 1 models. Toxicologic uncertainties include mammalian toxicities to combinations of piperonyl butoxide and adulticides and to inert ingredients in the formulated products. The addition of piperonyl butoxide to the adulticides increases the mosquito toxicity of the pyrethroids approximately 10-fold, but mammalian toxicity is not likely to be proportionally increased (Knowles 1991). Even if mammalian toxicity were increased 10-fold to the pyrethroids, RQs would still be well below levels of concern. Human exposures to solvents and other inert ingredients are likely to be low, resulting in low risks (NYCDOH 2001). Future research should be directed toward reducing toxicity and exposure uncertainties associated with mosquito adulticides. In addition, future assessments should address ecologic risks.

Comparing risks. Although it is difficult to compare the risks directly, several conclusions can be drawn by considering both human risks from exposure to WNV and insecticides used to control adult mosquitoes. In a situation where application of mosquito adulticides occurs because of known human cases of WNV, an adult human female may have at least a 3% probability of being infected by WNV. An adult female in that same area conservatively may have a 100% probability of being exposed to a particular mosquito adulticide. Her probability of exposure to the insecticide may be greater than WNV infection, but the consequences (i.e., the risks) of the exposures would be very different. Once infected with WNV, an adult human female has approximately a 20% probability of expressing clinical signs of illness (WN fever) and, depending on age, a 0.67% probability of expressing neurologic disease. Depending on the insecticide, her acute exposure would be 0.0415–15.76% of the RfD (0.0004–0.1576% of the NOAEL). Consequently, her acute risks from the insecticide would be lower than her acute risks from WNV. Subchronic insecticide risks would also be negligible (Table 4), whereas subchronic and chronic WNV risks (disease sequelae) would be greater. Therefore, once exposed to the insecticide (based on the tier 1 exposure assumptions from this study), the risk of any adverse health effects to the adult female would be negligible.

Results from our risk assessment and the current weight of scientific evidence (Currier et al. 2005; Karpati et al. 2004; NYCDOH 2001; O'Sullivan et al. 2005; U.S. EPA 2000c, 2005a, 2005b, 2005c) indicate that human-health risks from residential exposure to mosquito adulticides are very low and are not likely to exceed levels of concern. Further, by virtually any current human-health measure, the risks from infection by WNV exceed the risks from exposure to mosquito insecticides. Therefore, perceptions that human-health risks from the insecticides used to control adult mosquitoes are greater than the risks from WNV currently cannot be supported by current scientific evidence. Our results, and the results from other studies, should be used by the U.S. EPA, public health officials, and the general public to make better-informed decisions about risk–risk tradeoffs.

Correction

In the original manuscript published online, the acute air concentration for naled in Table 2 and the RQ ranges for acute inhalation exposures and acute subchronic dermal exposures were incorrect. These have been corrected here.

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SALT MARSH DIKING AND NUISANCE MOSQUITO PRODUCTION ON CAPE COD, MASSACHUSETTS

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ABSTRACT. Ecological surveys of mosquito breeding in diked and drained Cape Cod estuaries were conducted in 1982 and 1983. Production of the locally predominant nuisance species, *Aedes cantator*, was apparently enhanced in stagnant drainage ditches by acidification, caused by diking and drainage activities, which mediated the oxidation and dissolution of naturally-occurring pyrite to sulfate. High sulfate depressed pH and favored immatures of acid-tolerant mosquitoes by limiting fish and probably invertebrate predators. Breeding of *Ae. cantator* was restricted almost exclusively to acidified drainage ditches and occurred throughout the summer following rain events of at least 2.5 cm. Adults of this species comprised 72% (1982) and 56% (1983) of all nuisance mosquitoes caught in combination light/CO_2 traps set in or near the coastal flood plain throughout the summer. Current water quality and related mosquito problems associated with salt marsh diking and drainage strongly suggest that present water management methods are counterproductive for mosquito control and wetland preservation.

INTRODUCTION

Historic marsh management practices have often resulted in long term changes to wetland hydrology and ecology affecting both the production of nuisance insects and our ability to control their populations (Angerilli and Beirne 1980, Fleetwood et al. 1978, LaSalle and Knight 1974, Lesser et al. 1976, Vorgetts et al. 1980). Dikes and tide gates have been employed since at least the mid-nineteenth century to restrict tidal flow (from usually small estuaries) in an attempt to enhance agricultural use of highly organic flood plain soils. On Cape Cod, where the agricultural value of marshlands actually decreased after diking (Sterling 1976), dike maintenance has often been justified to reduce saline habitats for *Aedes sollicitans* (Walker) in wetlands that historically produced abundant mosquitoes. However, the identity of these historic pests is not recorded. Recent studies (Portnoy 1983) suggest that the combined effects of diking and subsequent ditch drainage may in fact increase the reproductive success of certain *Aedes* mosquitoes, especially *Ae. cantator* (Coq.).

This paper summarizes the results of mosquito surveys conducted in diked and drained outer Cape Cod estuaries during 1982 and 1983, relating larvae abundance and resultant adult nuisance levels to the quality of aquatic habitat.

STUDY AREAS

Most observations were made in the Herring River basin of Wellfleet (Fig. 1), an originally 445-ha salt marsh isolated by a dike from tidal flow (ca. 3.1 m tidal range) since 1908. Following the exclusion of seawater, salt marsh vegetation had been replaced by freshwater wetland species by the 1930's. With subsequent stream channelization and ditch drainage (begun about 1910, intensified in the 1930's and continued to the present for floodwater mosquito control), most of the basin's wetland habitat has been effectively drained, evidenced by the widespread invasion of upland shrubs and trees (Portnoy and Soukup 1982). Except during times of extremely heavy precipitation, surface water is restricted to the Herring River mainstream, tributary creeks and drainage ditches. Surface water quality varies seasonally with flow and biological activity, but is typically low in oxygen (20% saturation in ditches and 50% in main tributaries) and, especially in low-flow ditches, high in sulfate. Sulfate in solution, i.e.

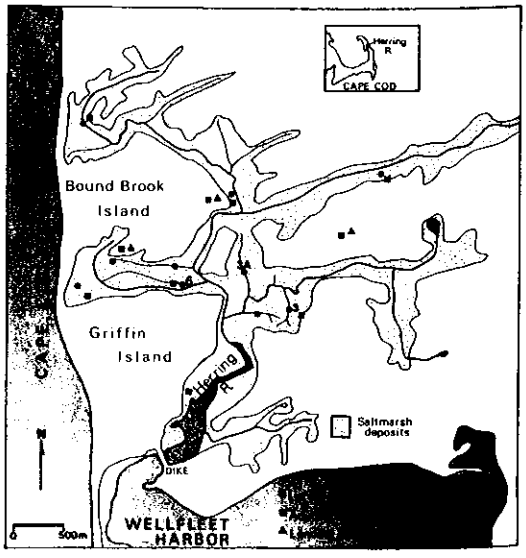

Fig. 1. The diked Herring River floodplain showing mosquito sampling stations.

sulfuric acid, severely depresses pH to ca. 3.0 in the poorly buffered water. Intensive drainage in the diked flood plain has allowed the aeration and oxidation of pyrites normally sequestered anaerobically in salt marsh sediments (M. A. Soukup and J. W. Portnoy, unpublished data) in a process that has been noted (Neely 1958, 1962; Edelman and Van Staveren 1958, Gosling and Baker 1980), but little appreciated. Ditches average 1–2 m wide, 1 m deep, and because they receive interstitial seepage through rather impermeable material, have a slow channel flow velocity which reaches a maximum of up to 5 $cm.s^{-1}$ in the central ditch channel following heavy rains.

Occasional observations of mosquito larval abundance, larval species composition and water quality were also made in Salt Meadow, Truro, another diked estuary about 11 km north of the Herring River. Salt Meadow, comprising about 150 ha of original salt marsh within its total 300-ha flood plain, was isolated from tidal flow about 1860 and thereafter has had a drainage history similar to that of the Herring River basin—with similar effects on plant succession and, from preliminary observations, water quality. Again, surface water is largely restricted to channelized streams and mosquito control drainage ditches.

METHODS

Semiweekly (1982) and weekly (1983) larval surveys were conducted from April through August at 10 stations throughout the Herring River flood plain. Specific sampling locations were chosen as typical of available larval habitat (surface water with little or no flow) for each physiographic sub-area of the coastal flood plain after initial surveys in 1981 (Fig. 1). I also sampled breeding habitat outside the flood plain on a weekly basis in both 1982 and 1983. Immatures were collected with a standard 1-pint (500-ml) dipper using a protocol intended to maximize dip counts by concentrating on highest larval/pupal densities within 15 m of each specific station. Water quality routinely included pH and conductivity, measured electrometrically at each station and date. Larval surveys in Salt Meadow were conducted irregularly in 1982 and weekly in 1983 using the same procedures.

Light traps (BioQuip EVS)[1] baited with 100 g dry ice were set one night (1800 hr to 0800 hr) weekly from early June through August to document emergence and to sample relative densities and species composition of adult mosquitoes at seven stations in the Herring River flood plain and at three nearby upland residences. Landing counts, conducted by exposing an observer's naked forearm for 10 min. at dusk, accompanied light trapping at the three residential sites which were located within 500 m of the flood plain. I did not sample adult mosquitoes in the vicinity of Salt Meadow in either year.

SURVEY RESULTS AND DISCUSSION

In both 1982 and 1983, *Ae. cantator* was the predominant nuisance mosquito breeding in the Herring River and Salt Meadow diked flood plains. Figure 2 describes larval densities at three representative Herring River stations regularly sampled in 1982 and 1983.

As suggested by Weaver and Fashing (1981), breeding appeared less affected by season than by changes in wetland water levels. In 1982, larvae were very abundant and widespread following record precipitation in May and June that inundated much of the diked flood plain; breeding continued throughout the 1982 summer with consecutive broods following rain

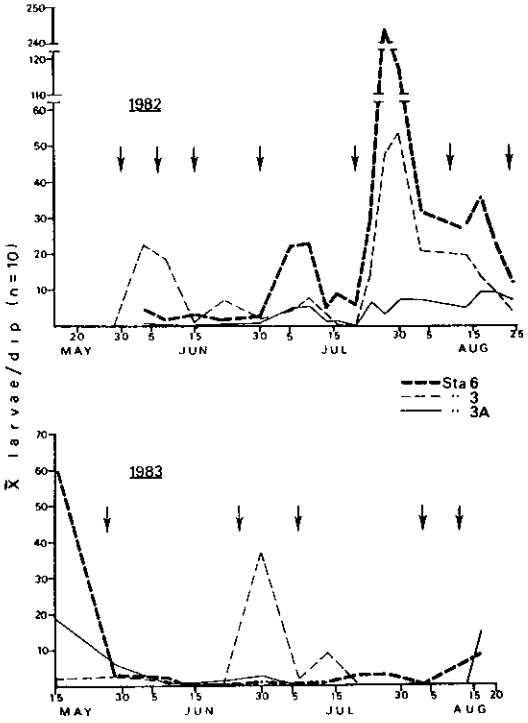

Fig. 2. Mosquito larvae densities at three typical acidified breeding sites in the Herring River basin during 1982 and 1983. Arrows (↓) indicate rain events of 2.5 cm or more.

[1] BioQuip Products, Santa Monica, CA 90406.

events of 2.5 cm or more. Low rainfall in spring 1983 resulted in no coastal marsh flooding and correspondingly low larval densities. Breeding was probably also suppressed in 1983 at specific sampling stations by periodic (but unreported) larvicide applications by the local mosquito control agency. Most breeding occurred in slow-flowing or stagnant drainage ditches. *Aedes cantator* immatures also predominated in ditches draining the Salt Meadow flood plain in 1982 and 1983.

Ditch water pH and conductivity for periods of high larvae counts (greater than 10 larvae/dip) are given in Table 1, demonstrating the extremely acidic conditions. High conductivity (total dissolved solids) is largely due to strongly acidifying sulfate ions, together with chloride; pH values of 3.2 correspond with sulfate concentrations of 200—700 ppm. The low pH in turn mobilizes toxic metals like aluminum, reaching as high as 49 ppm in pH 3.2 ditch water (M. A. Soukup and J. W. Portnoy, unpublished data). This combination of extremely low pH and high aluminum, toxic to fish at 0.3 ppm (Driscoll et al. 1980), severely limits larvivorous fish. Minnow traps placed in mosquito control drainage ditches throughout summer and fall never caught fish despite the frequent occurrence of *Lepomis gibbosus* and *Fundulus* spp. in the adjacent and confluent mainstream having better water quality. From casual observations, macro-invertebrates, including important mosquito predators (see Mathazan 1976) such as Dytiscidae, Hemiptera, and Odonata, are also scarce in acidic ditch water. The depression of invertebrate diversity and density in similarly high sulfate strip mine drainage is well documented (Roback and Richardson 1969, Bell 1971, Dills and Rogers 1974, Scullian and Edwards 1980). Correlations of invertebrate densities with water quality and *Ae. cantator* larval densities will be undertaken in the future.

Extensive surveys of outer Cape Cod wetland habitats in both 1982 and 1983 (unpublished data) revealed little *Ae. cantator* breeding outside the diked acidified flood plains.

The reproductive success of *Ae. cantator* was indicated by 1981 and 1982 observations of successful emergence (hatched puparia) and by adult densities in 1982 and 1983 trapping (Table 2 and Fig. 3) and landing count data (Fig. 3). This species comprised 52% (1982) and 49% (1983) of all females trapped, and 72% (1982) and 56% (1983) of all pest mosquitoes trapped. (Pest mosquitoes are defined here as those that commonly bite man in this area.) Casual observations since the summer of 1981 indicate that *Ae. cantator* is the predominant nuisance biter throughout the outer Cape Cod area.

The almost exclusive occurrence and observed success of *Ae. cantator* breeding in acidic ditches of diked flood plains, together with the absence of vertebrate and the rarity of invertebrate predators, suggest the primary source of the *Ae. cantator* nuisance. It is likely that *Ae. cantator* immatures, adapted to high conductivity and tolerant of low pH (MacGregor 1929), are selectively favored over pH-sensitive predators. In unaltered coastal marshes, *Ae. cantator* larvae are typically found in brackish (Magnarelli 1978) and high-ionic-strength freshwater at the salt marsh/upland ecotone (P. Slavin, personal communication). A genetically fixed preference for brackish habitats could be manifested in site selection by ovipositing adult fe-

Table 1. pH and conductivity at typical Herring River larvae sampling stations during periods of high larvae density (greater than 10 larvae per dip).

Date	pH	Conductivity (μmhos/cm) @ 25°C.	Date	pH	Conductivity
1982	Sta 3			Sta 6	
6–3	3.13	860	7–6	3.04	1470
6–8	3.59	480	7–9	3.06	1530
7–23	3.47	1495	7–23	3.24	1515
7–27	3.30	1836	7–27	3.30	1310
7–30	3.00	1628	7–30	3.20	1131
8–5	3.92	524	8–5	3.64	1305
8–13	3.30	1101	8–13	3.05	1363
8–17	3.27	1669	8–17	3.30	1434
1983	Sta 3A			Sta 6	
3–31	3.52	749	3–31	3.49	714
4–14	3.60	720	4–12	3.37	598
5–5	3.81	1122	4–20	3.48	696
5–13	3.85	768	5–5	3.48	1135
8–16	3.36	1200	5–13	3.96	738

Table 2. Summary and comparison of 1982 and 1983 mosquito trapping results in the Herring River Basin. Pest species included all *Aedes* spp. and *Coquillettidia perturbans*.

Species	Total females trapped (%) 1982	Total females trapped (%) 1983	Females/trap-night[+] 1982 (n = 145)	Females/trap-night[+] 1983 (n = 96)	Percent of pest species 1982	Percent of pest species 1983
Aedes cantator	10279 (52)	3547 (49)	70.9	37.0	72	56
Aedes canadensis	3437 (18)	2427 (34)	23.7	25.3	24	38
Aedes vexans	181 (1)	3 (T)	1.2	T	1	T
Aedes cinereus	128 (T)	63 (1)	0.9	0.7	1	1
Aedes excrucians	35 (T)	1 (T)	0.2	T	T	T
Aedes sollicitans	14 (T)	4 (T)	0.1	T	T	T
Aedes provocans	9 (T)	0 (0)	T	0	T	—
Aedes triseriatus	3 (T)	1 (T)	T	T	T	T
Culex spp.	4100 (21)	697 (10)	28.3	7.3	0	0
Culiseta minnesotae	1168 (6)	55 (1)	8.1	0.6	0	0
Culiseta melanura	46 (T)	73 (1)	0.3	0.8	0	0
Culiseta morsitans	1 (T)	2 (T)	T	T	0	0
Coquillettidia perturbans	228 (1)	307 (4)	1.6	3.2	2	5
Orthopodomyia signifera	1 (T)	0 (0)	T	0	0	—

[+] Significant change (decrease) for *Aedes cantator* only (t-test, $P<.05$).

males as well as in (and because of) enhanced survival of immatures.

Studies of two other salt marsh *Aedes*, *Ae. sollicitans* (Dixon 1947) and *Ae. taeniorhynchus* (Wied.) (McGaughey 1968), implicate sulfate as a powerful oviposition attractant. Abundant sulfate in both natural-brackish and altered acidic *Ae. cantator* habitats may be an important attractant for this species as well. Interestingly,

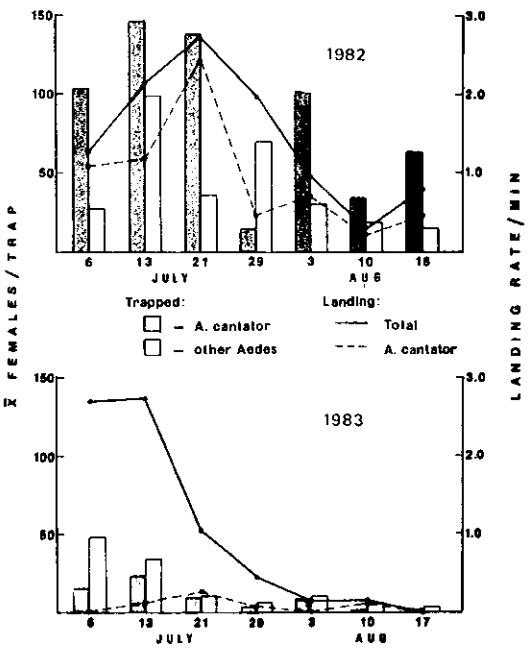

Fig. 3. Mean trap (n = 10) and landing (n = 3) counts of *Ae. cantator* vs. other biting mosquitoes in the Herring River area in 1982 and 1983.

immatures of more typically freshwater species, eg., *Ae. canadensis* (Theobald), *Ae. cinereus* (Meigen), and several *Culex* spp., occurred only in isolated and limited flood plain locations where sulfate, conductivity and acidity were low.

CONCLUSIONS

Although the development of extremely acidic conditions following salt marsh diking and drainage has been observed elsewhere (Edelman and Van Staveren 1958, Neely 1958, Gosling and Baker 1980), the overall significance to wetland functions (M. A. Soukup and J. W. Portnoy, unpublished data) and, in particular, to biting insect production is not well recognized. In view of recent observations, continued drainage in these diked salt marshes may be an inappropriate method of "source reduction" control. The consequent oxidation of pyrite acidifies and degrades water quality for larvivorous predators and selectively favors acid-tolerant *Ae. cantator* immatures.

More appropriate management of diked systems (Ferrigno and Jobbins 1968, J. Shisler, personal communication) would include the restoration of seawater flow to increase tidal flushing and to neutralize acidity in presently stagnant and acidic freshwater habitats. The re-creation of *Ae. sollicitans* breeding habitat is probably less of a concern to pest management because: 1) water quality problems that presently limit natural controls would be eliminated, and, 2) *Aedes sollicitans* production from adjacent outer Cape Cod salt marshes that are open to tidal flow is presently insignificant (Table 2 and unpublished data). Either little

suitable *Ae. sollicitans* habitat remains here or, more likely, local control agents experience greater success using source reduction methods more appropriately applied in undiked coastal marshes. Salt marsh diking on outer Cape Cod thus seems to be unjustified for the control of nuisance mosquitoes. In fact, diking probably generates a much less manageable pest control situation, and eliminates well-recognized (Nixon and Oviatt 1973) estuarine resources.

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Research Paper

Efficacy of Resmethrin Aerosols Applied from the Road for Suppressing *Culex* Vectors of West Nile Virus

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ABSTRACT

We determined whether aerosol applications of resmethrin, delivered from the road, suppress the reproductive activity of *Culex pipiens pipiens* and *Cx. restuans* mosquitoes in suburban sites located near Boston. Oviposition implies a prior blood-feeding event and hence a potential West Nile virus (WNV) transmission-related event. Droplet size, rate of delivery and meteorological conditions were monitored. The target populations proved to be fully susceptible to the insecticide that was used. The roads in the test sites generally gave adequate opportunity for insecticidal coverage. We found that the aerosol plume may have failed to contact the target mosquitoes and conclude that such insecticidal aerosols, delivered from the road, may not effectively reduce the force of transmission of WNV in our test sites. Key Words: Mosquito control—Adulticide—ULV—Resmethrin—*Culex pipiens pipiens*—*Culex restuans*—Arbovirus—West Nile virus. Vector-Borne Zoonotic Dis. 6, 117–127.

INTRODUCTION

The remarkable expansion of the range of West Nile virus (WNV) since its appearance in the United States in 1999 continues to challenge public health and mosquito abatement agencies (Roehrig et al. 2002). The various *Culex* mosquitoes that are regarded as the principal vectors of this pathogen are widely distributed in North America (Taylor et al. 1953, Hurlbut et al. 1956). Source reduction efforts frequently are directed against the aquatic stages of these vector mosquitoes. Catch basins, for example, are treated routinely with microbial or hormonomimetic insecticides (McCarry 1996, Siegel and Novak 1999). Interventions recommended by federal, state and local health authorities generally focus on the application of adulticides delivered from aircraft and road vehicles (Mount et al. 1996). Such "ultra-low volume" (ULV) applications of aerosols (Mount 1996) are widely used to protect residents of North America against the mosquito-borne encephalitides.

Large-scale applications of insecticidal aerosols became practical shortly after World War II, with the advent of potent insecticides and when the devices that had been used for generating naval smokescreens were "beaten into plowshares." The resulting thermal aerosol generators dispensed dilute insecticidal formulations with reasonable efficacy, and this methodology

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rapidly became widespread (Mount 1998). The technology permitting ULV applications of insecticide was developed during the 1950s (Knapp and Roberts 1965). Although the apparatus for dispensing such aerosols originally had been mounted on aircraft, such devices soon were adapted for use on ground-based vehicles, thereby becoming the "worldwide standard ground aerosol method of mosquito adulticiding . . . because of the inherent advantages over [high volume] aerosols" (Mount 1998). Although numerous field trials have demonstrated that insecticidal aerosols are lethal to caged mosquitoes (Mount 1998), few have monitored their impact on mosquitoes in nature. One such study demonstrated an 80% reduction of *Culex* species on the night after treatment, but concluded that a single application was probably inadequate for meaningful reduction of human risk of arboviral infection (Reiter et al. 1990). The general epidemiological impact of vehicle-mounted ULV insecticidal applications, therefore, remains to be determined.

It may be that risk of human infection by WNV can be reduced by aerosol formulations of insecticide applied from the road. Accordingly, we determined the impact of ULV applications of resmethrin on *Culex* mosquitoes in suburban sites in the vicinity of Boston. We used ovitraps to record the abundance of egg rafts deposited by *Culex pipiens pipiens* and *Cx. restuans* mosquitoes in the treated sites before and after aerosols were dispersed, as well as in sites in which no such aerosols were applied.

METHODS

Monitoring the Culex *spp. population*

The abundance of *Culex* mosquitoes was assessed by counting egg rafts deposited each night in arrays of ovitraps placed where aerosols were to be dispersed (Reiter 1986). Each ovitrap was a standard, black plastic tote box (54.0 × 40.0 × 12.7cm; Tablecraft Products Company, Inc, Gurnee, IL). The attractant was prepared by steeping 500 g of hay in 120 L of water for 7 days. Four liters of this infusion were placed in each pan several hours before sunset and renewed daily. Pans were spaced about 50 m apart. Egg rafts were counted each morning and a sample of 24 rafts transported to the laboratory and allowed to hatch. The resulting larvae were identified to species by examining the egg tooth and the surrounding cranial surface (Reiter 1986). Oviposition activity was monitored for a minimum of two days

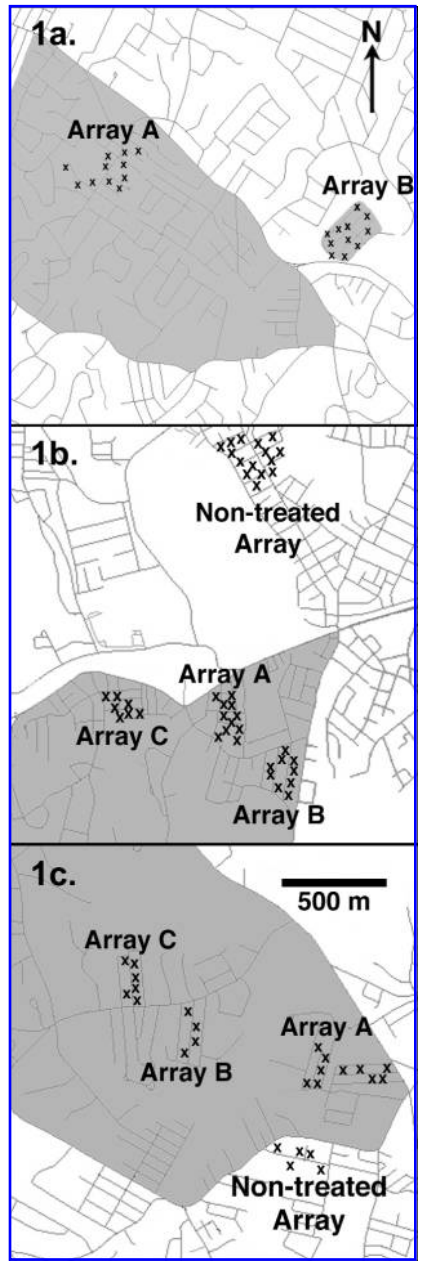

FIG. 1. The distribution of streets in the towns subjected to insecticidal treatment, the location of ovitraps (marked X), and the extent of the aerosol applications (shaded). (**a**) Burlington, MA. (**b**) Framingham, MA. (**c**) Tewksbury, MA.

prior to treatment and for up to two weeks thereafter.

Study sites

Six trials were completed in 2001–2002. Two were conducted in Burlington, eastern Massachusetts. The treatment area (Fig. 1a) included two arrays of ovitraps. Although many streets in the region are spaced irregularly, those where the ovitraps were set were spaced less than 30 m apart. Most of the houses were large, single-family homes, spaced well apart and set back from the road, each covering about 0.1 ha. Most were surrounded by lawns and gardens, including trees and shrubs of various sizes. Some house-lots included areas of dense secondary-growth forest.

Three trials were conducted in the nearby community of Framingham. The treatment area (Fig. 1b) included three arrays of ovitraps, all set along streets with similar layout and appearance. Although many houses were free standing, some were attached, multi-family units. Houses were spaced about 10m apart, and had a somewhat less affluent appearance.

A third array of ovitraps was set in the nearby community of Tewksbury (Fig. 1c). The street layout, lot-size and landscaping characteristics resembled that of Burlington. For comparison, we monitored arrays of ovitraps in non-treated areas in Framingham, Tewksbury (Fig. 1b,c), and Cambridge. Non-treated sites in Framingham and Tewksbury were similar to those that were treated.

Insecticide application

Resmethrin aerosols (Scourge® 18% A.I.+54% PBO; Bayer Environmental Science, Montvale, NJ) were generated from an 18-hp Grizzly® Cold Aerosol PDS ULV generator (Clarke Mosquito Control, Roselle, IL). Droplet size was determined with an AIMS hot wire portable droplet counter (Model DC-III; KLD Laboratories, Inc., Huntington Station, NY). The sprayer was adjusted to generate a surface mean diameter of 16–18 μm and to release the aerosol 45 degrees above the horizon. Flow rate was adjusted to 89 mL/min for trials 1–3 and 106 mL/min for trials 4–6. Windspeed ranged from 1km/h to 8 km/h, and air temperature was above 18°C when the application commenced. Insecticide was applied at three of four U.S.Environmental Protection Agency (EPA)–approved treatment rates (1.12, 1.96, and 7.85 g/ha). Spraying commenced 0.5 h after astral sunset and ended within about 2 h. Wind direction, speed, ambient temperature and relative humidity were recorded immediately

TABLE 1. RATE OF RESMETHRIN TREATMENT, LOCATION AND DATE OF EACH TRIAL

					Dates			
			Traps		*Observations*			
Trial	*Resmethrin, g/ha*	*Town*	*Array*	*No.*	*Begun*	*Ended*	*Aerosol applied*	*Year*
1	1.12	Burlington	A	14	15 Jul	26 Jul	18 Jul	2001
"	"	"	B	12	"	"	"	"
2	"	"	A	14	30 Jul	16 Aug	2 Aug	"
"	"	"	B	12	"	"	"	"
3	1.96	Framingham	A	11	5 Aug	3 Sep	13 Aug	"
"	"	"	B	9	"	"	"	"
4	7.85	Tewksbury	A	10	4 Sep	13 Sep	6 Sep	"
"	"	"	B	4	"	"	"	"
"	"	"	C	7	"	"	"	"
"	"	"	Untreated	5	"	"	None	"
5	"	Framingham	A	11	8 Jul	25 Jul	16 Jul	2002
"	"	"	B	9	"	"	"	"
"	"	"	C	10	"	"	"	"
6	"	"	A	8	5 Aug	12 Aug	6 Aug	"
"	"	"	B	7	"	"	"	"
"	"	"	Untreated	15	"	"	None	"

prior to each application. Local weather data were compared to data recorded by an automated meteorological station operated by the National Weather Service in the nearby town of Bedford, MA.

Insecticide susceptibility

Larval *Cx. p. pipiens* and *Cx. restuans* obtained from egg rafts collected at the Cambridge and Burlington, MA, sites were sent to CDC Malaria Branch where they were reared to adulthood and tested by bioassay and enzyme analysis. Adults were held for 1 h in bottles containing residues of 20 μg of resmethrin, 43 μg of deltamethrin, or 25 μg of cypermethrin. All mosquitoes died after 30 min of exposure, indicating full pyrethroid susceptibility.

Statistical analysis

Multi-variate regression analysis was performed using a statistical software program (MINITAB, release 13.31, MINITAB Inc., State College, PA). Variables compared in this analysis included (1) whether or not insecticide had been applied, (2) days elapsed since such an application (limited to four days) and (3) mini-

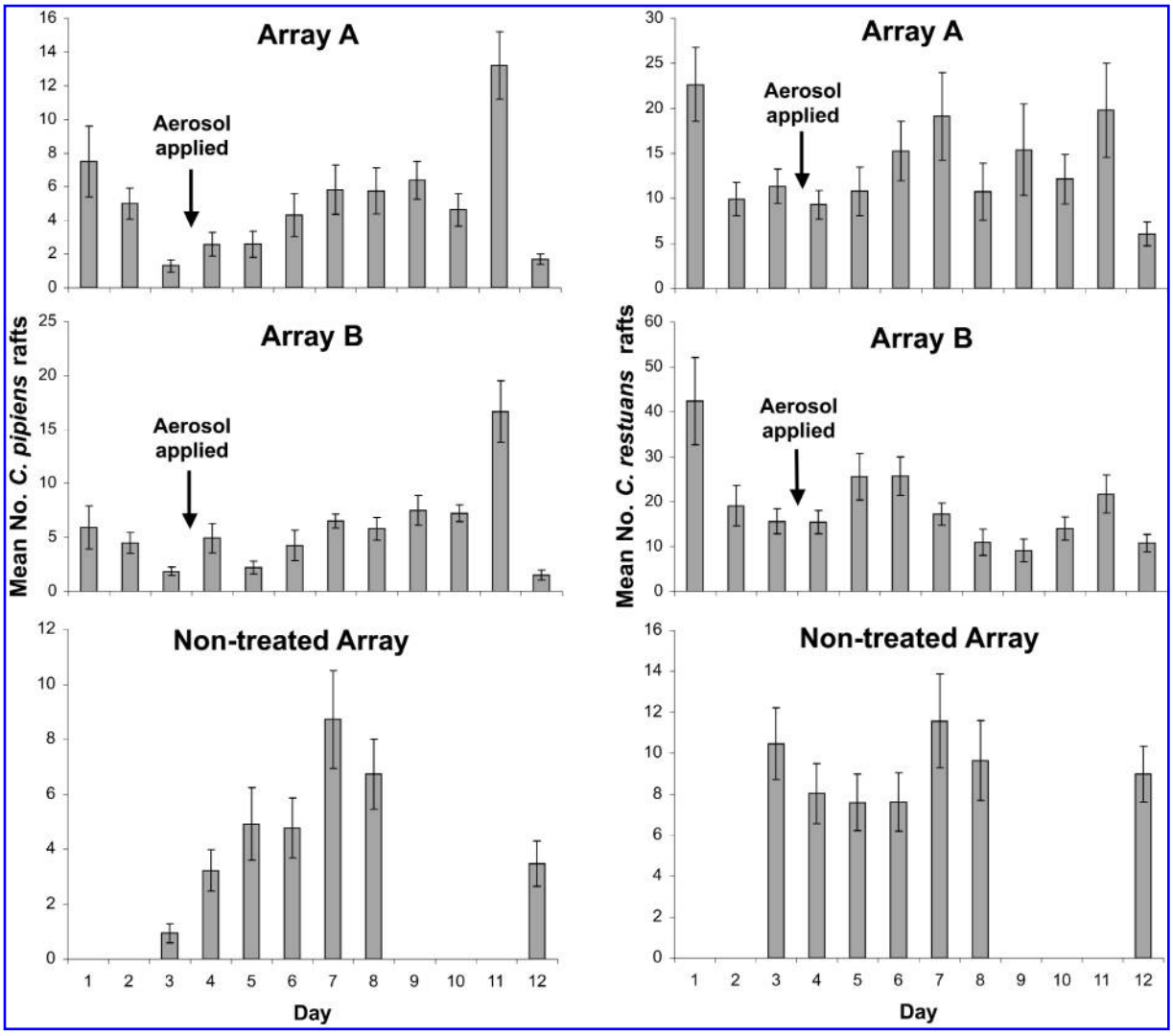

FIG. 2. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 1 following an aerosol application of resmethrin at 1.12 g/ha in Burlington, MA, during 15–26 July 2001. Non-treatment observations were recorded at sites in Cambridge, MA, and Jamaica Plain, MA. No collections made on days 1, 2, 9, and 11.

mum ambient temperature on each of these days. A p value of <0.05 was considered to be significant.

RESULTS

The first trial was conducted in Burlington during mid-July of 2001 (Table 1). Insecticide was applied at the minimum specified rate of 1.12 g/ha. This evaluation was based on an array of traps set near the center of a larger treated site (Array A) and another that covered the entire treated area (Array B). Insecticide was applied on the fourth evening after observations commenced. During the 12-day period of observation, the number of egg rafts deposited in each array fluctuated synchronously (Fig. 2), with *Cx. restuans* depositing more eggs than *Cx. p. pipiens*. About as many eggs were deposited by *Cx. p. pipiens* ($p < 0.073$) and *Cx. restuans* ($p < 0.400$) before the insecticidal application as after, more or less in parallel with that in the non-treated sites.

Trial 2 was conducted in the same sites two weeks later using the same application rate, and continued for 18 days (Table 1). Insecticide was applied on the fourth evening. Oviposition frequency was relatively constant in both arrays of traps, with the exception of a depression during the second night after treatment (Fig. 3). Rain fell heavily throughout that night,

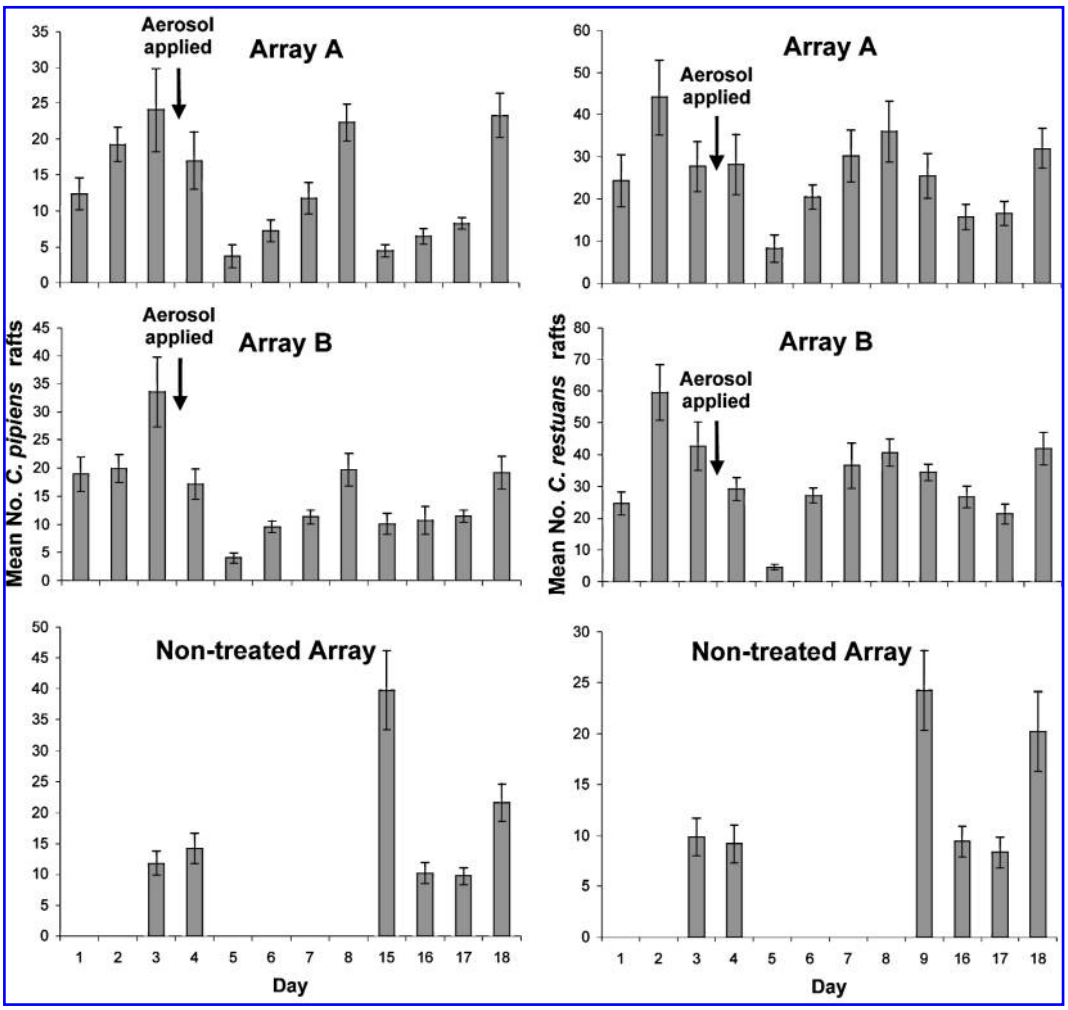

FIG. 3. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 2 following an aerosol application of resmethrin at 1.12 g/ha in Burlington, MA, during 30 July to 16 August 2002. Non-treatment observations were recorded at sites in Cambridge, MA, and Jamaica Plain, MA. No collections made on days 1, 2, and 5–15.

and observations in the non-treatment sites were interrupted during this critical period. Somewhat more *Cx. p. pipiens* eggs were deposited in treated sites before the intervention than after ($p < 0.001$). No such effect was observed on *Cx. restuans* oviposition activity ($p < 0.266$).

In Trial 3, conducted in Framingham during August of 2001, the application rate was increased to 1.96 g/ha (Table 1) with treatment on the seventh evening after observations commenced. Oviposition activity rose after the treatment in parallel with that in the non-treatment sites (Fig. 4). Only a minimal effect by the aerosol application on *Cx. p. pipiens* was evident ($p < 0.046$). A significant, yet transient reduction in oviposition by *Cx. restuans* was noted ($p < 0.003$).

Trial 4 was conducted in Tewksbury early in September of 2001. Insecticide was applied during the third evening of the trial at the maximum rate specified on the label, which was four times greater than in Trial 3 (Table 1). For comparison, a nearby non-treated site was monitored, and three arrays of traps were set within the treated neighborhoods. This aerosol application, too, appeared not to have affected the reproductive activity of *Cx. p. pipiens* ($p < 0.593$) or *Cx. restuans* ($p < 0.879$; Fig. 5).

Trial 5 was carried out in Framingham during mid July of 2002. Insecticide was again applied at the maximum allowable rate, nine days after the first observation. Somewhat fewer egg rafts of *Cx. p. pipiens* ($p < 0.013$) and of *Cx. restuans* ($p < 0.034$) were deposited after the treatment than at other times (Fig. 6).

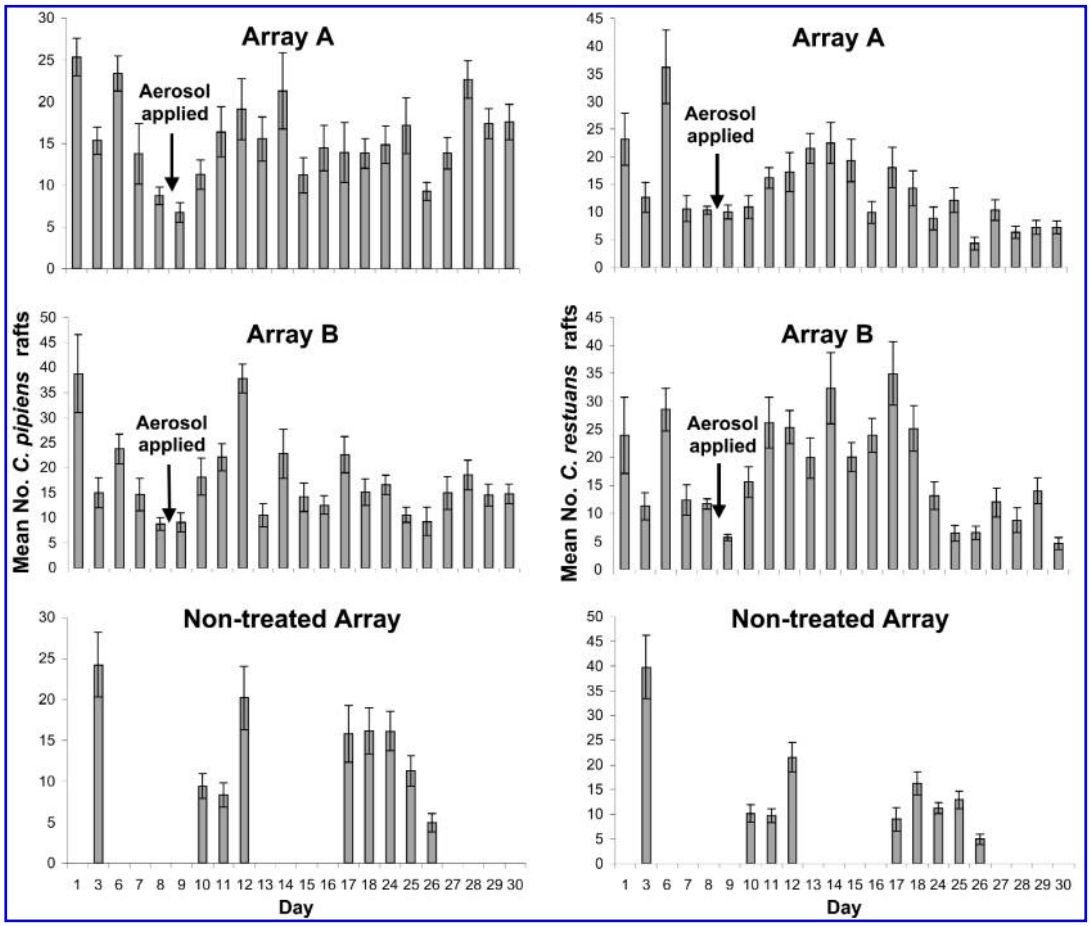

FIG. 4. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 3 following an aerosol application of resmethrin at 1.96 g/ha in Framingham, MA, during 5 August to 3 September 2002. Non-treatment observations were recorded at sites Cambridge, MA, and Jamaica Plain, Ma. No collections made on days 1, 2, 6–9, 13–16, and 27–30.

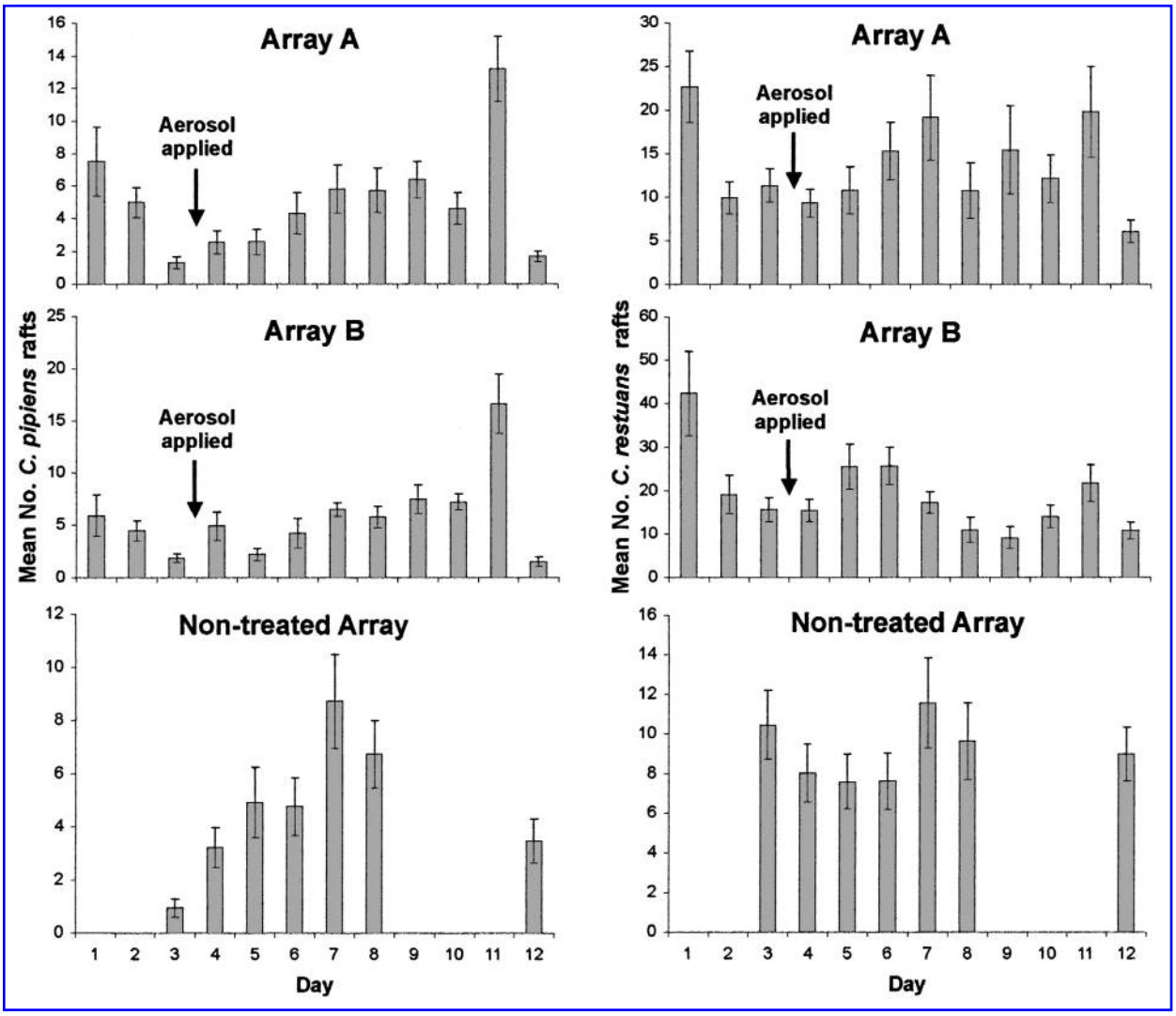

FIG. 5. Deposition of egg rafts in three arrays of ovitraps (designated A, B, and C) by *Cx. p. pipiens* and *Cx. restuans* in trial 4 following an aerosol application of resmethrin at 7.85 g/ha in Tewksbury, MA, during 4–13 September 2002. Non-treatment observations were recorded in Tewksburg, MA. No collections made on days 1 and 5–7.

In Trial 6, conducted in Framingham early in August of 2002, treatment was on the second evening, and was again at the maximum permitted rate. No reduction in the number of egg rafts of *Cx. p. pipiens* ($p < 0.295$) or of *Cx. restuans* ($p < 0.346$) was noted (Fig. 7).

DISCUSSION

The emergence of WNV in North America calls for interventions to protect the public health. Source reduction by sanitary and larvicidal measures receives much attention, but the preventive efficacy of such measures has not been demonstrated. Although many communities rely on adulticidal aerosols, this approach also awaits critical evaluation. Spraying from the road may reduce the density of "bridge vectors" that can carry infection from the avian cycle to human hosts; but our results suggest that this is not the case for the two kinds of mosquitoes that are considered important in enzootic transmission (Kilpatrick 2005). Moreover, a model of short-term reductions of adult mosquitoes on transmission suggests that the impact and sustainability required to reduce the force of transmission may be unattainable in practice (Newton and Reiter 1992).

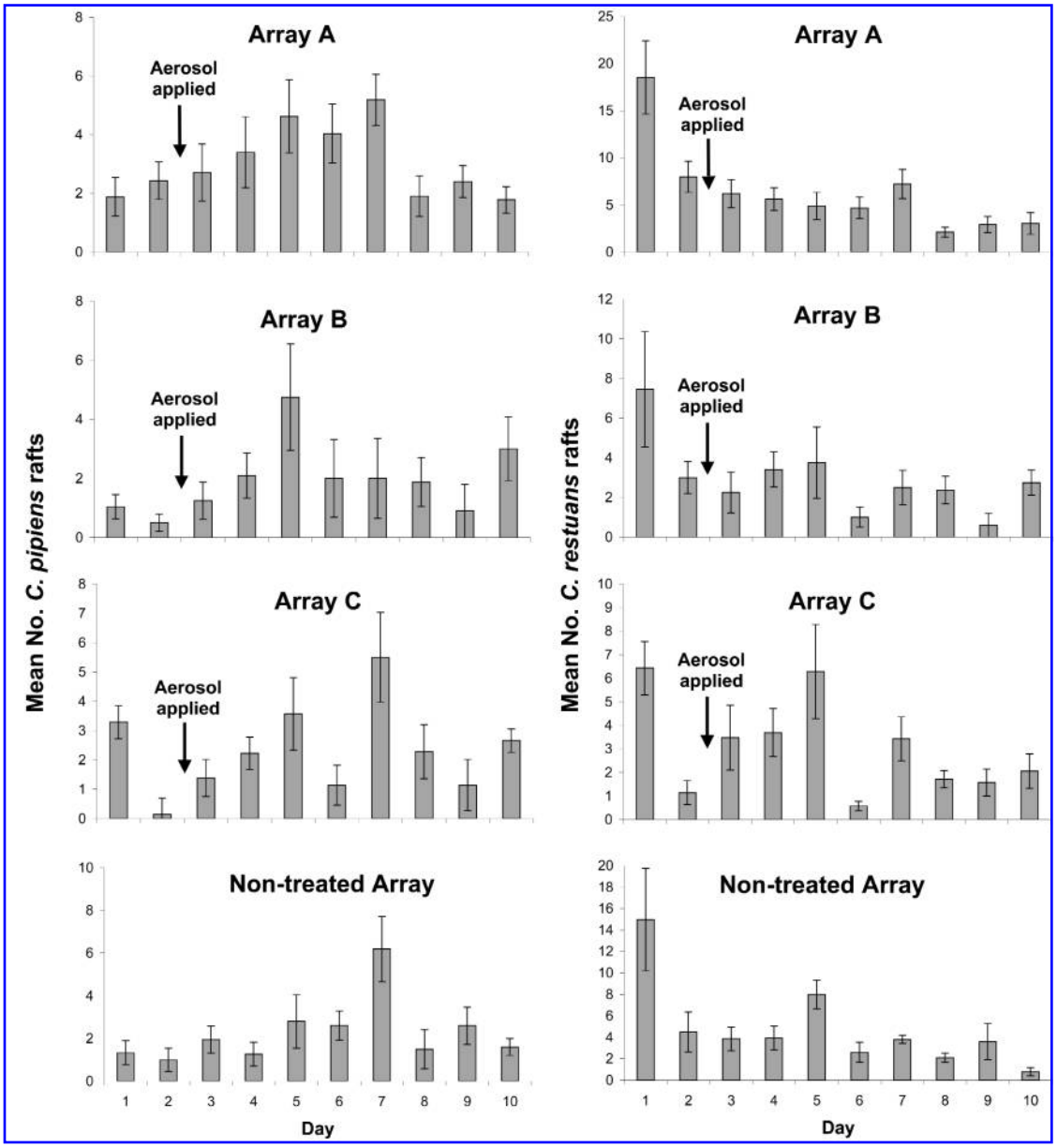

FIG. 6. Deposition of egg rafts in three arrays of ovitraps (designated A, B, and C) by *Cx. p. pipiens* and *Cx. restuans* in trial 5 following an aerosol application of resmethrin at 7.85 g/ha in Framingham, MA, during 8–25 July 2002. Non-treatment observations were recorded at sites in Cambridge, MA. No collections made on days 1, 2, 4–7, 12–14, and 16.

Factors that may affect the impact of an insecticidal aerosol applied from road vehicles include insecticidal insusceptibility, inadequate application rate, inadequate or excessive wind speed, inappropriate air temperature, lack of a suitable temperature inversion, physical obstacles to drift, an irregular network of roads, and the timing of treatments in relation to flight activity by the target species (Mount 1998).

In our study, droplet size and flow rate met EPA specifications. Insecticide was applied when ambient temperatures were 15–24°C. Wind conditions ranged from 1 to 8 km/h. Careful attention was paid to vehicle speed. Treatments were made under a variety of conditions. Application rates covered the full range specified by the U.S. EPA, and the target mosquitoes were fully susceptible to the-

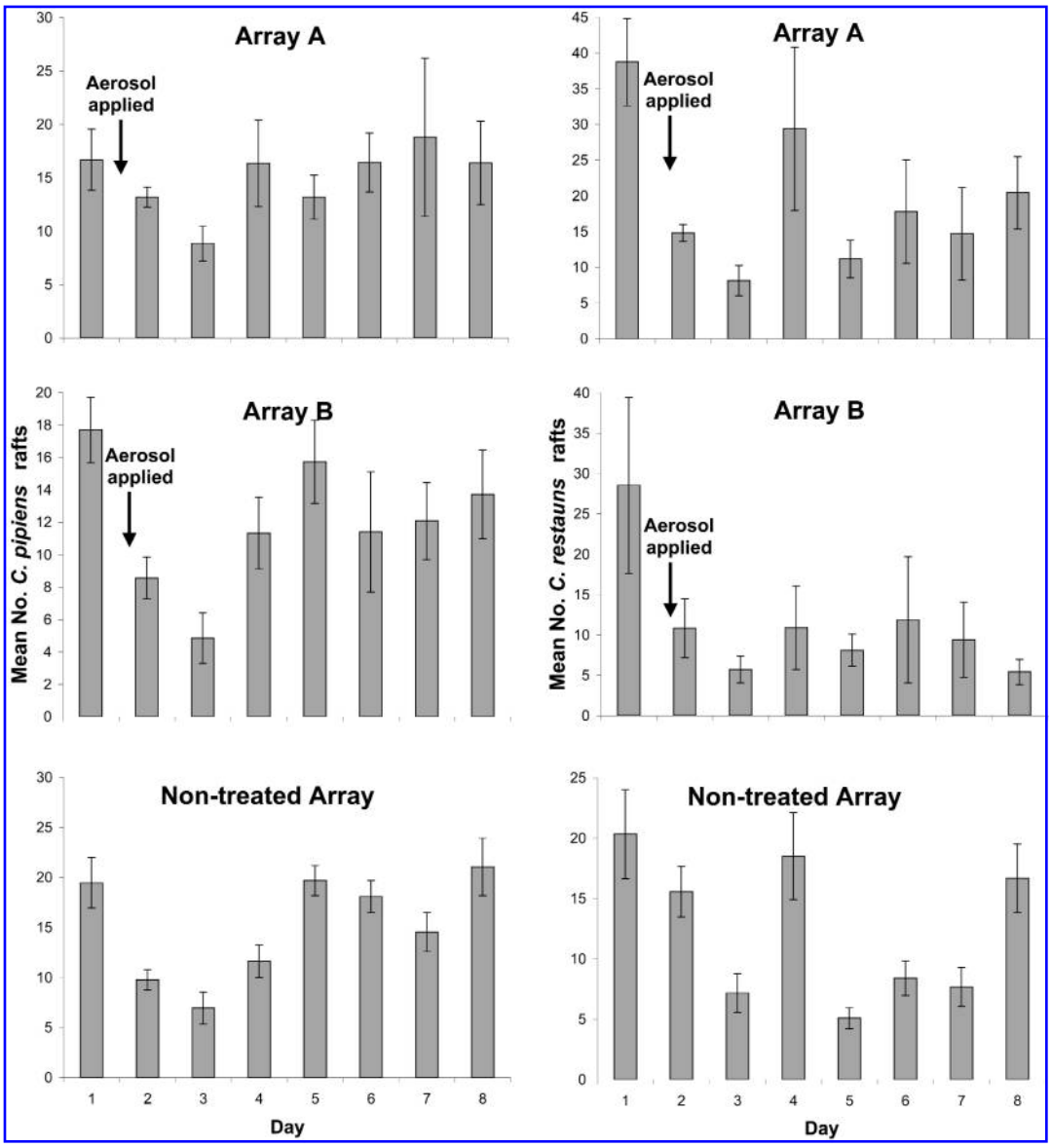

FIG. 7. Deposition of egg rafts in two arrays of ovitraps (designated A and B) by *Cx. p. pipiens* and *Cx. restuans* in trial 6 following an aerosol application of resmethrin at 7.85 g/ha in Framingham, MA, during 5–12 August 2002. No collections made on days 4–7.

insecticide. In summary, we tested the method under a variety of circumstances, and made every attempt to optimize conditions, yet none of our treatments had any demonstrable impact.

The effective swath width for ULV treatments is about 90 m. The streets in our test areas were all less than 90 m apart and were selected to give maximum opportunity for optimum coverage. As in many parts of New England, however, a large proportion of nearby streets are spaced irregularly, thus limiting area-wide uniformity of aerosol coverage.

Culex p. pipiens, is said to fly up to 0.91 km in a single night (Schreiber et al. 1988). Spray evaluations may therefore be confounded by immigration if the monitoring devices are located too close to the periphery of the treatment area. Because the dimensions of residential areas in our studies are characteristic of the region, our trapping arrays were clustered near the center of the

treatments, several were nearer than 0.9 km to the non-treated periphery.

Buildings, trees, dense brush and other obstacles affect drift (Mount et al. 1966) and create eddies that may result in variable rates of exposure of target mosquitoes (Reiter et al. 1990, Reiter and Gubler 1997). In addition, our studies (Lepore et al. 2004) confirmed other published evidence (Main et al. 1966, Anderson et al. 2004) that *Cx. p. pipiens* and *Cx. restuans* quest in the tree canopy, rather than near the ground; the temperature inversions required for optimum drift may reduce effective delivery of aerosol to such sites.

The timing of insecticidal applications is crucial because aerosols mainly are effective against mosquitoes when they are in flight. We observed that questing and oviposition by *Cx. p. pipiens* and *Cx. restuans* peaked about two hours after sunset, and declined steadily thereafter until sunrise (T.J. Lepore unpublished data). Our applications were targeted for the two hours of maximum activity, and may have missed a major portion of mosquitoes that were active later in the night.

In a previous study, one of us (P.R.) reported an 80% reduction of populations of *Cx. p. pipiens* and *Cx. restuans* in Memphis, TN. The insecticide, methods of evaluation and the target mosquito species were essentially the same. A relative lack of obstacles to drift may account for this difference: in Memphis, plots were two to five times larger than in New England, houses were separated by extensive lawns and open spaces, with little shrubbery or other ground vegetation. In addition, the regular grid plan of streets was optimal for even coverage. By contrast, in our treatment areas, houses were closer together, and vegetation was abundant, often dense.

We find that ULV applications of resmethrin had little or no impact on the *Culex* vectors of WNV, even at maximum permitted rates of application. A model simulating the major outcomes of such treatments indicates that they are unlikely to reduce the force of transmission of such an arbovirus (Newton and Reiter 1992). We conclude that insecticidal aerosols dispersed from the road may not effectively reduce the force of transmission of WNV.

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COMPARATIVE EFFECTIVENESS OF THREE ADULT MOSQUITO SAMPLING METHODS IN HABITATS REPRESENTATIVE OF FOUR DIFFERENT BIOMES OF CALIFORNIA

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ABSTRACT. The effectiveness of New Jersey (NJ) light, dry ice baited, and gravid female traps for collecting adult mosquitoes was compared at representative habitats in the Coachella, San Joaquin, and Sacramento valleys and the Los Angeles basin of California. The NJ light traps effectively sampled *Anopheles freeborni, Culex tarsalis, Psorophora columbiae,* and several *Aedes* when abundance was high in rural areas with minimal competitive illumination. Dry ice-baited encephalitis virus surveillance or CDC style traps collected significantly more females of most species at most localities than did NJ light traps, regardless of background illumination. The Cummings modification of the Reiter gravid female trap baited with a bulrush (*Schoenoplectus*) infusion was the best method for collecting *Culex pipiens* complex females in most habitats. In the Los Angeles basin, gravid traps baited with bulrush infusion collected, on average, 4.5 times more *Culex quinquefasciatus* females than did traps baited with the Reiter infusion. The bulrush infusion in combination with the Cummings trap design seemed to provide resting site cues and collected males as well as empty and bloodfed females. Mosquito surveillance programs in California should include the systematic operation of dry ice-baited and gravid female traps to improve surveillance sensitivity for selected species in appropriate habitats.

KEY WORDS Adult collection methods, California, *Culex tarsalis, Culex pipiens* complex

INTRODUCTION

The accurate measurement of adult mosquito abundance is critical in evaluating control programs and in forecasting encephalitis virus activity. California mosquito and vector control districts currently participate in a statewide mosquito surveillance program that provides weekly Mosquito Occurrence Reports averaging the catch of adult *Culex tarsalis* Coquillett and other mosquito genera in New Jersey (NJ) light traps (Mulhern 1942) within urban, suburban, and rural habitats. Originally, light traps were deployed to monitor *Cx. tarsalis* abundance in relation to enzootic virus transmission activity (Longshore 1960, Loomis and Meyers 1960) and replaced labor intensive resting catches in natural shelters and red boxes (Loomis and Green 1959) as a method of monitoring adult abundance.

The NJ light trap samples nocturnal, phototactic mosquitoes such as *Cx. tarsalis* with minimal collection effort; however, catch size is reduced markedly by competing light sources (Milby and Reeves 1989). Decreased sensitivity of NJ light traps in conjunction with the progressive urbanization of California have led most control agencies to incorporate supplemental sampling methods into their mosquito surveillance programs, including CDC (Sudia and Chamberlain 1962) or encephalitis virus surveillance (EVS) (Pfuntner 1979) traps augmented with dry ice (hereafter, CO_2 traps) and CDC (Reiter 1987) or Cummings (Cummings 1992) gravid female traps baited with alfalfa infusion (Reiter 1983). The CO_2 traps rely on a CO_2 chemotaxis by host-seeking females and therefore are less influenced by background illumination. Gravid female traps baited with the standard alfalfa infusion (alfalfa, lactalbumin, and yeast) attract mostly *Culex quinquefasciatus* Say and are most effective in urban habitats (Reisen and Pfuntner 1987, Reisen and Meyer 1990).

The current research compared the effectiveness of NJ light, CO_2, and gravid female traps for sampling *Cx. tarsalis* and other potential vector and pest mosquitoes in representative habitats in 4 different ecological areas of California. This research extends the results of previous trap effectiveness studies (e.g., Hayes et al. 1958, Milby et al. 1978, Meyer et al. 1984, Reisen and Pfuntner 1987, Milby and Reeves 1989, Reisen et al. 1990) that compared these and other methods in specific habitats. An increasing human population encroaching on formerly rural areas, expanded and effective mosquito control, and improved flood and irrigation water management have combined to markedly alter the landscape of California as well as mosquito abundance and diversity (Reeves and Milby 1989). Our research evaluated the effectiveness of 3 sampling methods for monitoring mosquito abundance in this altered landscape.

MATERIALS AND METHODS

During 1997, representative habitats were studied concurrently in 4 major biomes of California:

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the southern Coachella Valley in Riverside County, the Bakersfield area of the southern San Joaquin Valley in Kern County, the Sacramento Valley in Sacramento and Yolo counties, and the Los Angeles basin in Orange County. Selection of localities within each biome was based on the presence of a NJ light trap and habitat type. Habitats included urban premises, suburban residences, urban parks, riparian corridors, managed marshland, irrigated agriculture, and golf courses. Differentiation between urban and suburban areas was based on the extent of vegetation and human population density. Urban parks often were located along water courses but usually were isolated by surrounding housing areas, whereas rural riparian habitat presented an extensive vegetative continuum transecting agricultural habitat. All agriculture was irrigated and included row crops (Coachella, San Joaquin, Sacramento), date orchards (Coachella), citrus (San Joaquin), grapes (San Joaquin, Sacramento), rice (Sacramento), and pasture (Sacramento).

Three trap types were compared at 8–10 localities within each of the 4 biomes on 1 night per week from July through October 1997. Standard NJ light traps were hung at 2.4 m (8 ft.) height and fitted with 25-watt incandescent or 5-watt fluorescent lamps (Sacramento and Yolo counties) that yielded comparable brightness (≈250 lumens). The CO_2 traps (CDC style in Coachella, San Joaquin, and Sacramento; EVS style in Los Angeles) were operated concurrently without lights at fixed standards of 1.5 m height positioned within 50 m of the NJ light trap and baited with dry ice presented in modified 3.7-liter (1-gal.) insulated cans (≈2.5 kg) or styrofoam holders (Coachella, ≈1.5 kg). All gravid traps were constructed following the revised design of Reiter (1987) upgraded by Cummings (1992) and were operated at fixed locations on the ground within 50 m of the NJ light trap. Gravid traps were baited with 1-wk-old bulrush (*Schoenoplectus* [=*Scirpus*]) infusion consisting of 4.5 g of chopped and dried bulrush stems per liter of tap or well water (Walton and Workman 1998). The bulrush infusion attracted a broad range of species (especially *Culex stigmatosoma*) in field tests (Walton and Workman 1998) and attracted *Cx. tarsalis* in laboratory experiments (J. Millar, personal communication).

Attractiveness of the bulrush infusion was compared with the original alfalfa infusion (Reiter 1983) at 8 sites in the Los Angeles basin during a 10-wk period. Traps were operated at 2 fixed positions at the same locality within 50 m of each other. Infusions were prepared fresh each week and rotated between positions to account for any spatial effects.

Mosquitoes were returned to the laboratory where they were sorted to species, sex, and female abdominal condition (empty, bloodfed, gravid) and counted. Abdominal conditions were comparable with Sella's (1920) stages 1, 2–5, and 6–7, respectively. Counts were transformed by ln(y + 1) to control the variance and normalize the distribution. Separate multiway classification analyses of variance (ANOVA) were conducted on data from each of the 4 biomes, with collection method and habitat as main effects (GLM procedure for fixed effects with 1st-order interactions, NCSS software version 6.0.22). Data initially were blocked by weeks (n = 10 − 12), but time accounted for <5% of the total variance at all biomes and was deleted in the final ANOVAs. In the Los Angeles basin, counts from gravid traps baited with alfalfa infusion were not included in the general ANOVA but rather were compared with counts from traps baited with the bulrush infusion in separate ANOVAs with media, position, and habitat as main effects. Throughout, posteriori Newman–Keuls multiple comparison tests were used to group means that were not significantly different ($P > 0.05$). Means in text and tables were backtransformed or geometric means expressed as numbers per trap per night. Initially, product moment correlations were calculated for transformed and untransformed female counts of the abundant species collected in NJ light, CO_2, and gravid traps at each biome. However, ln(y + 1) transformations did not appreciably improve the fit and only untransformed values are shown.

RESULTS

Fauna: A total of 37,601 female and 4,787 male mosquitoes comprising 19 species in 5 genera were collected by all methods (Table 1). *Culex pipiens* complex species were most abundant and widespread, followed by *Cx. tarsalis*. *Culex pipiens* complex specimens from the Sacramento and San Joaquin valleys were a mixture of *quinquefasciatus* and *pipiens* genotypes, whereas specimens from the Los Angeles basin and Coachella Valley south of the Tehachapi Mountains were only *quinquefasciatus* (Urbanelli et al. 1997). *Aedes* species were associated with irrigated pasture and were most abundant in the Coachella and Sacramento valleys. *Anopheles freeborni* Aitken was abundant in the rice growing areas of the Sacramento Valley. *Culiseta incidens* (Thomson) was abundant in residential areas in the Los Angeles basin and Sacramento Valley. *Psorophora columbiae* (Dyar and Knab) was found only in the Coachella Valley. Statistical analyses were limited to the abundant species (total ≥200) indicated by bold font in Table 1.

Coachella Valley: Gravid traps collected significantly more *Cx. quinquefasciatus* females and males than did either NJ light traps or CO_2 traps (Table 2). The interaction between method and habitat was significant; catch in CO_2 traps was greatest in agricultural habitat, whereas catch in gravid traps was greatest in agricultural and golf course habitats. Catch of *Cx. tarsalis* females was greatest in CO_2 traps in suburban and agricultural habitats. Interestingly, gravid traps collected significantly more

Table 1. Total female (F) and male (M) mosquitoes of each species collected by all methods during 1997.[1]

Species collected	Coachella		San Joaquin		Los Angeles		Sacramento		Total	
	F	M	F	M	F	M	F	M	F	M
Aedes melanimon	0	0	27	0	0	0	1,075	33	1,102	33
Aedes nigromaculis	0	0	53	0	0	0	11	3	64	3
Aedes vexans	121	0	0	0	0	0	384	8	505	8
Anopheles hermsi	0	0	0	0	110	3	0	0	110	3
Anopheles franciscanus	1	0	5	1	6	4	27	8	39	13
Anopheles freeborni	0	0	4	0	0	0	**4,776**	**1,008**	4,780	1,008
Anopheles punctipennis	0	0	0	0	0	0	2	0	2	0
Culex apicalis	0	0	0	0	2	0	0	0	2	0
Culex boharti	0	0	0	0	1	1	0	0	1	1
Culex erythrothorax	1	0	2	0	142	0	186	0	331	0
Culex pipiens/quinquefasciatus	**3,054**	**1,235**	**9,415**	**801**	**2,879**	**224**	**2,956**	**2,912**	18,304	2,564
Culex stigmatosoma	4	0	12	1	79	0	140	23	235	24
Culex tarsalis	**1,771**	195	**1,924**	50	**394**	5	**7,784**	**865**	11,877	1,115
Culex thriambus	0	0	0	0	61	0	0	0	61	0
Culiseta incidens	0	0	0	0	93	1	95	27	188	28
Culiseta inornata	0	0	0	0	1	0	2	0	3	0
Culiseta particeps	0	0	0	0	1	0	0	0	1	0
Psorophora columbiae	**8,509**	30	0	0	0	0	0	0	8,509	30
Total	13,461	1,460	11,442	853	3,769	238	17,438	2,266	37,601	4,787

[1] Data in bold were used for trap evaluations.

Table 2. Backtransformed mean number of adult mosquitoes per trap night, Coachella Valley.[1,2]

Species	Sex	Method	Habitat URB (TN = 72)	SUB (TN = 71)	AGRI (TN = 141)	GOLF (TN = 72)	Mean (TN = 356)
Culex quinquefasciatus	F	NJLT	0.09	0.00	0.22	0.22	0.14c
		CO2T	1.65	3.87	6.18	2.11	3.12b
		Gravid	4.87	5.23	7.97	14.53	7.46a
		Mean	1.57bc	2.15abc	3.28ab	2.89ab	2.41
Cx. quinquefasciatus	M	NJLT	0.00	0.00	0.15	0.11	0.01b
		CO2T	0.00	0.33	0.01	0.11	0.13b
		Gravid	1.15	1.69	5.97	8.70	3.45a
		Mean	0.29b	0.53b	1.07a	1.29a	0.75
Culex tarsalis	F	NJLT	0.00	0.57	1.25	0.09	0.41c
		CO2T	0.22	8.78	5.88	0.01	2.09a
		Gravid	0.22	2.13	1.86	0.32	0.86b
		Mean	0.15b	2.63a	2.53a	0.01b	1.01
Cx. tarsalis	M	NJLT	0.00	0.18	0.67	0.00	0.19a
		CO2T	0.00	0.13	0.00	0.00	0.00b
		Gravid	0.00	0.42	0.92	0.00	0.31a
		Mean	0.00c	0.24b	0.49a	0.02c	0.18
Psorophora columbiae	F	NJLT	0.00	0.35	6.67	0.28	0.91a
		CO2T	0.13	0.38	12.00	1.09	1.55a
		Gravid	0.00	0.00	0.01	0.00	0.00b
		Mean	0.00b	0.23b	3.74a	0.39b	0.70

[1] Means within each species/sex followed by the same letter were not significantly different by N-K multiple range test ($P > 0.05$).
[2] URB = urban, SUB = suburban, AGRI = agriculture, GOLF = golf course, TN = total trap nights for 3 methods at 10 sites for 12 wk, F = female, M = male, NJLT = New Jersey light trap, CO2T = CO_2 trap, Gravid = gravid trap.

Cx. tarsalis females than did NJ light traps. No significant difference was found between the catches of male *Cx. tarsalis* in NJ light and gravid traps, both of which were greatest in agricultural habitat. No significant difference was found in the catches of female *Ps. columbiae* between the NJ light and CO_2 traps, both of which were significantly greatest in agricultural habitat. Practically all *Aedes vexans* (Meigen) were collected by CO_2 traps in agricultural habitat (data not shown).

San Joaquin Valley: Catch of both *Cx. quinquefasciatus* females and males was greatest in gravid traps operated in agricultural and riparian habitats (Table 3). Catch of *Cx. tarsalis* females was greatest in CO_2 traps operated in riparian and agricultural habitats. The riparian locality along the Kern River was adjacent to citrus orchards irrigated with a drip system that pooled excessive water at the tree bases, providing extensive larval habitat.

Los Angeles basin: Gravid traps baited with bul-

Table 3. Backtransformed mean number of adult mosquitoes per trap night, San Joaquin Valley.[1,2]

Species	Sex	Method	Habitat URB (TN = 99)	SUB (TN = 66)	AGRI (TN = 131)	RIP (TN = 33)	Mean (TN = 329)
Culex quinquefasciatus	F	NJLT	0.01	0.00	0.63	0.48	0.27c
		CO2T	3.77	1.56	58.97	6.40	7.60b
		Gravid	10.79	12.24	28.80	5.04	11.95a
		Mean	2.91c	2.27c	13.27a	6.15b	4.21
Cx. quinquefasciatus	M	NJLT	0.00	0.01	0.01	0.35	0.12b
		CO2T	0.00	0.00	0.02	0.46	0.12b
		Gravid	2.02	1.72	5.49	2.35	2.66a
		Mean	0.48b	0.43b	0.91a	0.87a	0.66
Culex tarsalis	F	NJLT	0.01	0.00	0.74	0.17	0.97b
		CO2T	0.33	0.22	10.35	32.12	3.96a
		Gravid	0.11	0.16	0.17	0.42	0.21c
		Mean	0.17c	0.12c	1.85b	6.20a	1.28

[1] Means followed by the same letter within species/sex were not significantly different by the N-K range test ($P > 0.05$).
[2] URB = urban, SUB = suburban, AGRI = agriculture, RIP = riparian, TN = total trap nights for 3 methods at 10 sites for 11 wk, F = female, M = male, NJLT = New Jersey light trap, CO2T = CO_2 trap, Gravid = gravid trap.

Table 4. Backtransformed mean number of adult mosquitoes per trap night, Los Angeles basin.[1,2]

Species	Sex	Method	Habitat URB (TN = 144)	MAR (TN = 93)	PARK (TN = 36)	Mean (TN = 273)
Culex quinquefasciatus	F	NJLT	0.14	0.09	0.86	0.32c
		CO2T	4.09	0.81	0.19	1.22b
		Gravid	24.87	2.68	20.43	11.68a
		Mean	4.32a	0.94c	2.62b	2.34
Cx. quinquefasciatus	M	NJLT	0.01	0.28	0.19	0.17b
		CO2T	0.01	0.00	0.00	0.00b
		Gravid	1.53	0.27	1.61	1.03a
		Mean	0.43a	0.17b	0.46a	0.35
Culex tarsalis	F	NJLT	0.14	0.16	0.67	0.30b
		CO2T	0.40	5.29	1.63	1.85a
		Gravid	0.33	0.13	0.12	0.19b
		Mean	0.28b	1.02a	0.70a	0.64

[1] Means followed by the same letter within each species/sex were not significantly different by the N-K range test ($P > 0.05$).
[2] URB = urban, MAR = marsh, PARK = urban park, TN = total trap nights for 3 methods at 8 sites for 12 wk, F = female, M = male, NJLT = New Jersey light trap, CO2T = CO_2 trap, Gravid = gravid trap.

rush infusion collected the most female *Cx. quinquefasciatus* in urban and park habitats in Orange County; CO_2 traps were most effective in urban habitats (Table 4). Similar to females, most *Cx. quinquefasciatus* males were collected in gravid traps operated in urban and park habitats. In contrast, most *Cx. tarsalis* females were collected by CO_2 traps in marsh habitat. Gravid traps baited with bulrush infusion collected significantly more *Cx. quinquefasciatus* females and males and *Cs. incidens* females than did gravid traps baited with the alfalfa infusion (Table 5). Position effects were significant for *Cx. quinquefasciatus* males but not females, indicating that males were influenced more by trap placement than were females. Habitat effects also were significant in the ANOVAs for *Cx. quinquefasciatus* but not for *Cs. incidens.*

Sacramento Valley: Catch size of *An. freeborni* females was significantly greatest in CO_2 and NJ light traps operated in agricultural habitat (Table 6). Catch of *An. freeborni* males was greatest in NJ light traps, followed by gravid traps. Catch of *Cx. pipiens* females and males was greatest in gravid traps operated in urban and suburban residential habitat. Catch of *Cx. tarsalis* females was greatest in CO_2 traps, followed by NJ light traps, operated in agricultural habitats.

Female abdominal condition: The 3 sampling methods targeted different portions of the female *Culex* population (Table 7). The NJ light and CO_2 traps collected mostly empty females (i.e., no visible blood or eggs), whereas gravid traps collected more gravid females. Proportionately more gravid female *Cx. pipiens* than *Cx. tarsalis* were collected in gravid traps. In contrast, most (>95%) females of species such as *Aedes* and *Psorophora* were unfed, regardless of the sampling method used (data not shown).

Correlation analysis: Correlation coefficients among sampling methods were calculated over both time and space for species collected abundantly by more than 1 method (Table 8). Catch of *Cx. tarsalis* females collected by NJ light trap was significantly correlated ($P < 0.01$) with females collected by CO_2 trap in all biomes except for urban Los Angeles basin, where catch was low overall. Catch of *Cx. tarsalis* in gravid traps was correlated with NJ light traps at 2 of 4 sites and with CO_2 traps at 1 of 4 sites. In contrast, catches of *Cx. pipiens* in gravid and CO_2 traps were correlated at 3 of 4 sites, but NJ light trap data was correlated with CO_2 and gravid traps at only 1 each of 4 sites. Although abundance of *An. freeborni* varied significantly among methods (Table 6), patterns measured by all 3 methods were well correlated over time and space in the Sacramento Valley. Similarly, the catch of

Table 5. Backtransformed mean number of females collected in gravid traps baited with bulrush or alfalfa infusions.[1]

Species	Sex	Bulrush (TN = 94)	Reiter (TN = 87)	P[2]
Culex quinquefasciatus	F	11.01	2.44	<0.001
	M	1.09	0.31	<0.001
Culiseta incidens	F	0.35	0.01	<0.001

[1] TN = trap nights, F = female, M = male.
[2] P, means significantly different.

Table 6. Backtransformed mean number of adult mosquitoes per trap night, Sacramento Valley.[1,2]

Species	Sex	Method	Habitat URB (TN = 88)	SUB (TN = 77)	AGRI (TN = 239)	Mean (TN = 404)
Anopheles freeborni	F	NJLT	0.01	1.59	6.26	1.71a
		CO2T	0.27	1.52	7.97	2.06a
		Gravid	0.35	0.00	0.98	0.40b
		Mean	0.22c	0.89b	4.05a	1.27
An. freeborni	M	NJLT	0.00	0.37	1.72	0.57c
		CO2T	0.00	0.00	0.00	0.00c
		Gravid	0.21	0.00	0.72	0.28a
		Mean	0.08b	0.11b	0.69a	0.26
Culex pipiens	F	NJLT	0.65	0.69	0.41	0.58c
		CO2T	1.28	5.41	1.68	2.39b
		Gravid	23.06	9.98	2.26	8.51a
		Mean	3.49a	3.92a	1.31b	2.71
Cx. pipiens	M	NJLT	0.24	0.98	0.30	0.47b
		CO2T	0.00	0.00	0.00	0.00c
		Gravid	1.86	0.78	0.19	0.82a
		Mean	0.53a	0.54a	0.16b	0.40
Culex tarsalis	F	NJLT	0.87	0.84	6.33	1.93b
		CO2T	2.10	12.78	36.83	10.74a
		Gravid	2.46	0.16	1.17	1.05c
		Mean	1.72b	2.08b	7.49a	3.13
Cx. tarsalis	M	NJLT	0.19	0.37	2.62	0.81a
		CO2T	0.00	0.00	0.00	0.00c
		Gravid	0.63	0.00	0.84	0.46b
		Mean	0.26b	0.13b	0.90a	0.40

[1] Means followed by the same letter within each species/sex were not significantly different by the N-K range test ($P > 0.05$).
[2] URB = urban, SUB = suburban, AGRI = agriculture, TN = total trap nights for 3 methods at 10 sites for 12 wk, F = female, M = male, NJLT = New Jersey light trap, CO2T = CO_2 trap, Gravid = gravid trap.

Ps. columbiae in NJ light traps was correlated significantly ($P < 0.01$) with catch in CO_2 traps ($r = 0.591$) in the Coachella Valley; too few were collected in gravid traps for analysis.

Model II regression equations then were calculated for significantly correlated pairs of data to estimate the catch in NJ light traps (NJLT) from catch in CO_2 traps (CO2T). These regressions may be useful in sampling programs replacing NJ light with CO_2 traps and having historical data limited to NJ light traps. For *Cx. tarsalis* equations for each biome were as follows: Coachella, NJLT = 0.876 + 0.139CO2T, $R^2 = 0.138$; San Joaquin, NJLT = 0.179 + 0.103CO2T, $R^2 = 0.615$; and Sacramento, NJLT = 4.780 + 0.050CO2T, $R^2 = 0.163$. With the exception of the San Joaquin Valley, these regressions explained <20% of the variability observed among NJ light trap counts. In the Los Angeles basin, the regression coefficient did not differ significantly from 0 ($P > 0.05$).

DISCUSSION

Catch sizes by different sampling devices varied markedly among species and habitats. The NJ light traps worked well for *An. freeborni, Cx. tarsalis,* and *Ps. columbiae* in agricultural and riparian habitats where competing illumination was minimal. Catch size at NJ light traps was greater in agricultural habitats in the Sacramento Valley than in the Coachella and San Joaquin valleys. Differences in catch were not attributed to incandescent vs. fluo-

Table 7. Percent abdominal condition composition of *Culex tarsalis* and *Culex pipiens* complex females collected at 4 biomes by 3 sampling methods.[1]

Method	*Cx. tarsalis* *n*	GR	BF	MT	*Cx. pipiens* complex *n*	GR	BF	MT
NJLT	1,224	7.7	1.1	91.2	249	11.2	1.6	87.1
CO2T	9,804	0.7	0.5	98.8	9,071	0.4	0.7	98.8
GRAV	240	56.5	9.5	34.0	1,475	77.1	6.3	16.6

[1] NJLT = New Jersey light trap, CO2T = CO_2 trap, GRAV = gravid trap, GR = gravid, BF = bloodfed, MT = empty.

Table 8. Correlations over both time and space among untransformed counts of females per trap night.

Species and site	df	Trap comparison[1] 1 vs. 2	1 vs. 3	2 vs. 3
Culex tarsalis				
Coachella	115	0.28*	0.37*	0.54*
San Joaquin	108	0.78*	−0.05	0.15
Sacramento	108	0.40*	0.34*	0.18
Los Angeles	92	−0.06	0.02	−0.02
Culex pipiens complex				
Coachella	115	−0.02	0.17	0.33*
San Joaquin	108	0.09	0.44*	0.36*
Sacramento	108	0.40*	0.16	0.12
Los Angeles	92	0.09	0.16	0.51*
Anopheles freeborni				
Sacramento	108	0.78*	0.75*	0.71*

[1] 1 = New Jersey light trap; 2 = CO_2 trap; 3 = gravid trap.
* $P < 0.05$.

rescent lamp brightness (Barr et al. 1960) because both lamps purportedly output comparable lumens. Expanded use of security lighting at houses in many agricultural areas and the encroachment of suburban housing areas with home and street lighting have produced an "urban glow" and provided extensive competing background illumination (Milby and Reeves 1989). In addition, improved water management and mosquito control may have reduced mosquito population size in many areas of California. In combination, these factors reduced the catch in NJ light traps in many areas of the Coachella and San Joaquin valleys and essentially eliminated their effectiveness in the Los Angeles basin. Low catch size by NJ light traps throughout southern California during the current study agreed well with data trends summarized in unpublished Mosquito Abundance Reports during the past 10 years.

The CO_2 traps circumvented problems associated with competing background illumination and collected comparable or significantly greater numbers of all phototactic species than did NJ light traps, even in rural areas. The CO_2 traps also were advantageous in that they collected large numbers of *Cx. pipiens* complex females. Both NJ light and CO_2 traps sampled the same portion of the female population, collecting predominantly empty females. Previous studies in the Central Valley developed ratios between the catch size in NJ light and CO_2 traps in different habitats to estimate adult mosquito catch in NJ traps from that in CO_2 traps (Milby et al. 1978, Milby and Reeves 1989). We used linear regression to predict the number of *Cx. tarsalis* females per NJ light trap night from females per CO_2 trap night. However, these equations explained <20% of the variability in NJ light trap counts at 3 of 4 sites and generally were not considered useful in placing abundance measured by CO_2 traps into a historical context. Semi-log or log-log transformations did not markedly improve the R^2 values, indicating that the relationship between these 2 methods was linear over time and space within biomes.

Gravid traps baited with bulrush infusion collected the greatest numbers of *Cx. pipiens* complex females and males, especially in urban situations. The bulrush infusion also performed well in rural agricultural areas, where the alfalfa infusion previously was markedly less attractive and unable to compete with natural oviposition sites (Reisen and Pfuntner 1987, Reisen and Meyer 1990). The bulrush infusion collected significantly more females than did the alfalfa infusion in 3 habitats in Orange County and collected a greater diversity of species. Unfortunately, the bulrush infusion failed to collect large numbers of *Cx. tarsalis* gravid females when compared with concurrently operated CO_2 traps. Considering that the gonotrophic cycle of *Cx. tarsalis* is about 3 days long and that daily survivorship averages about 0.8 per day in most habitats (Reisen and Reeves 1990), then if equally effective, gravid traps should have collected about half the number of females as CO_2 traps, an effectiveness never achieved in the current study.

Interestingly, the gravid trap also collected males and nongravid females of several species that seemed to be responding to cues associated with resting sites, such as decaying *Schoenoplectus* odors, a plume of elevated humidity created as the fan moved air over the infusion, and/or the color of the black pan used to hold the infusion.

The present results indicated that the statewide mosquito surveillance program, which is based solely on NJ light trap data, should be expanded into an integrated mosquito surveillance program that utilizes the most sensitive sampling methods to measure the relative abundances of target species in specific habitats. Gravid traps should be used in urban and suburban environments to monitor *Cx. pipiens* complex species. The NJ light traps should be replaced by CO_2 traps in urban and suburban habitats and in those agricultural and rural areas where background illumination precludes the effective operation of NJ light traps. Because of the low sampling effort required and the phototactic response of the encephalitis virus vector species, *Cx. tarsalis* and *Aedes melanimon,* NJ light traps could be retained in rural areas of the Sacramento Valley where trap operation remains effective.

Additional research will be required to ensure adequate sensitivity and standardization. The CO_2 traps currently in use throughout California vary considerably in design (motor size, diameter, propeller configuration), light use, and dry ice presentation (pelletized or block ice placed in insulated cans with or without holes or in styrofoam holders). In addition, the frequency of operation and pattern of deployment should be optimized through conditional simulation experiments. The bulrush infusion improved catch size and species diversity in

gravid traps over the alfalfa infusion but still did not collect sufficient numbers of *Cx. tarsalis,* the target of most surveillance and control programs in California. Improvement of oviposition attractants will be necessary before this trap can replace other sampling devices for most mosquito species.

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Delinquent Mortgages, Neglected Swimming Pools, and West Nile Virus, California

William K. Reisen, Richard M. Takahashi, Brian D. Carroll, and Rob Quiring

Adjustable rate mortgages and the downturn in the California housing market caused a 300% increase in notices of delinquency in Bakersfield, Kern County. This led to large numbers of neglected swimming pools, which were associated with a 276% increase in the number of human West Nile virus cases during the summer of 2007.

Although West Nile virus (WNV) (family *Flaviviridae*, genus *Flavivirus*) has remained epidemic in California since its arrival in 2003 (*1*), the cascade of events enabling local outbreaks remains poorly understood. WNV is amplified enzootically among several passeriform bird species within concurrent rural and urban cycles and is tangentially transmitted to humans by several *Culex* mosquito species (*2*). *Culex tarsalis* is the primary rural vector whose abundance relies on the availability of surface water created by precipitation and agricultural irrigation, whereas members of the *Cx. pipiens* complex are urban vectors whose abundance is dependent on underground drainage systems, wastewater, or anthropogenic peridomestic sources (*3*). Surveillance data useful in tracking WNV risk include temperature anomalies, mosquito abundance and infection rate trends, sentinel chicken seroconversions, dead bird reports and necropsy results, and the numbers of reported equine and human cases. Each of these factors are assigned a risk score, averaged, and ranked in terms of overall risk from 1 (normal season) to 5 (epidemic conditions) (*4*).

The Study

An outbreak with 140 laboratory-confirmed human cases of WNV was centered in the Bakersfield area of Kern County, California, during 2007 (incidence = 17.5/100,000 population). This case cluster formed the WNV epicenter for California during 2007, was the largest mosquito-borne encephalitis virus outbreak in Kern County since the 1952 epidemic of western equine encephalitis virus (*5*) and represented a 205%–280% increase in the numbers of confirmed WNV cases observed since 2004 (*6*). The 2007 outbreak was unanticipated on the basis of surveillance data. Winter and spring weather was exceptionally dry (40% of expected rainfall) and hot (mean March–June temperatures ranged from 0.5°C–2.0°C above 30-year normal values). Rural *Cx. tarsalis* populations remained below 5-year averages (–32% to –76% of average during weeks 19–29) because of decreased rainfall, snow pack, and water allotments to agriculture. The Kern River, which flows through Bakersfield, remained mostly dry during spring and summer; key bird species decreased in abundance because of the drought (overall catch of free-ranging birds in 2007 was 31% of catch at the same traps during 2006) and the previous negative effect of WNV infection; and surviving birds in key species had high herd immunity to WNV (house finch WNV seroprevalence = 22%, n = 182; western scrub jay = 44%, n = 27) acquired during previous seasons.

Despite these findings, the infection incidence in *Cx. pipiens quinquefasciatus* increased rapidly to 18.5 females/1,000 mosquitoes in June 2007 at traps within Bakersfield, a month earlier than observed in previous summers (online Appendix Figure 1, available from www.cdc.gov/EID/content/14/11/1747-appF1.htm). With reduced competition from house finches and predation on nestlings by western scrub jays, house sparrow populations increased dramatically. This expanding population was dominated by hatching year birds, had limited protective immunity (4.1%, n = 311), and comprised 23% of 124 WNV-positive dead birds reported by the public. Early season high risk of WNV infection in birds was followed closely by human cases, and this and several other case clusters of high incidence stimulated an emergency appropriation of $6.2 million by the Governor's Office of the State of California to enhance surveillance and mosquito control.

Careful examination of service requests for mosquito control made to the Kern Mosquito and Vector Control District (KMVCD) and an aerial survey of Bakersfield showed an extensive number of green or neglected pools, most of which were producing mosquitoes. The likely reasons for neglected pools are the adjustable rate mortgage and associated housing crises in Kern County and throughout California, which have led to increased house sales, notices of delinquency of payment, declarations of bankruptcy and home abandonment. Kern County was especially affected (Figure 1), with a 300% increase in notice of delinquency in the spring quarter of 2007 compared with that of 2006. Associated with home abandonment was the expanding number of neglected swimming pools, Jacuzzis (hot tubs), and ornamental ponds. As chemicals deteriorated, invasive algal blooms created green swimming pools that were

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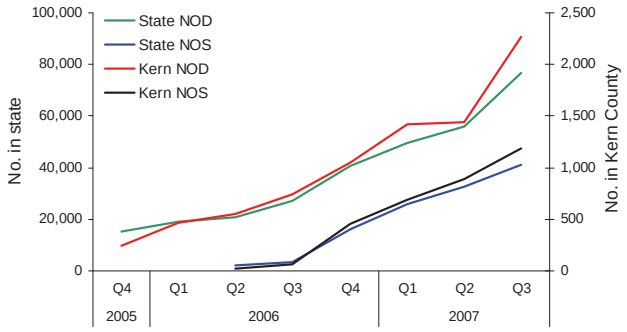

Figure 1. Notice of delinquency (NOD) and notice of sale (NOS) for homes in Kern County and California by quarter (Q) per year, 2005–2007.

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By law, all swimming pools or properties with pools have to be surrounded by 2-m high fencing and gates that must be locked when the homeowner is not present. These locked fences provided a formidable obstacle for mosquito control personnel to overcome for surveillance and treatment. Public awareness of this problem has been enhanced by education programs and media information, and local residents have begun to notify the KMVCD and other local agencies about neglected pool problems. Alarmingly, during 2008, many of these unmaintained pools previously positive for *Cx. p. quinquefasciatus* were now occupied by *Cx. tarsalis*, a more competent vector of WNV than *Cx. p. quinquefasciatus* (*7,8*). Collections of immature mosquitoes from 31 neglected pools taken during February–August 2008 produced 8,978 emerging adults, of which 59% were *Cx. tarsalis* and 41% were *Cx. p. quinquefasciatus*. Ongoing surveillance continues to monitor the extent of this problem in Kern County and throughout California and its affect on the ongoing WNV epidemic.

Figure 2. Aerial photograph of a representative Bakersfield, California, neighborhood taken during August 2007. Red arrows indicate neglected or green swimming pools. Letters (F, G, H, J) are photographic reference points.

Conclusions

Anthropogenic landscape change historically has facilitated outbreaks of pathogens amplified by peridomestic vectors such as *Cx. pipiens* complex mosquitoes and associated commensals such as house sparrows. The recent widespread downturn in the housing market and increase in adjustable rate mortgages have combined to force a dramatic increase in home foreclosures and abandoned homes and produced urban landscapes dotted with an expanded number of new mosquito habitats. These new larval habitats may have contributed to the unexpected early season increase in WNV cases in Bakersfield during 2007 and subsequently have enabled invasion of urban areas by the highly competent rural vector *Cx. tarsalis*. These factors can increase the spectrum of competent avian hosts, the efficiency of enzootic amplification, and the risk for urban epidemics.

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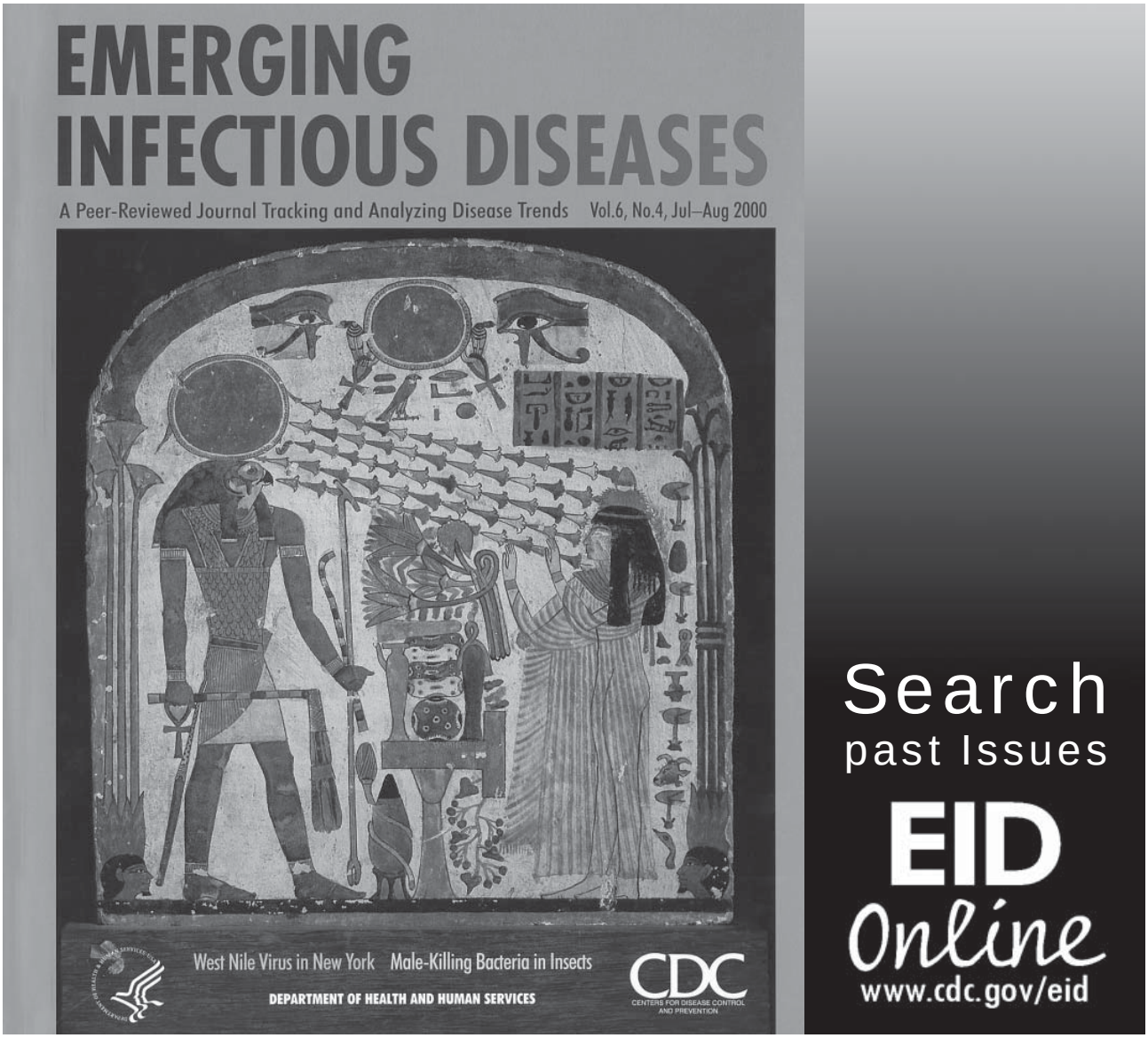

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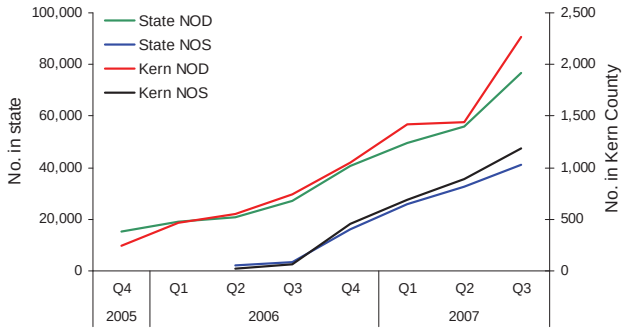

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Figure 2. Aerial photograph of a representative Bakersfield, California, neighborhood taken during August 2007. Red arrows indicate neglected or green swimming pools. Letters (F, G, H, J) are photographic reference points.

Conclusions

Anthropogenic landscape change historically has facilitated outbreaks of pathogens amplified by peridomestic vectors such as *Cx. pipiens* complex mosquitoes and associated commensals such as house sparrows. The recent widespread downturn in the housing market and increase in adjustable rate mortgages have combined to force a dramatic increase in home foreclosures and abandoned homes and produced urban landscapes dotted with an expanded number of new mosquito habitats. These new larval habitats may have contributed to the unexpected early season increase in WNV cases in Bakersfield during 2007 and subsequently have enabled invasion of urban areas by the highly competent rural vector *Cx. tarsalis*. These factors can increase the spectrum of competent avian hosts, the efficiency of enzootic amplification, and the risk for urban epidemics.

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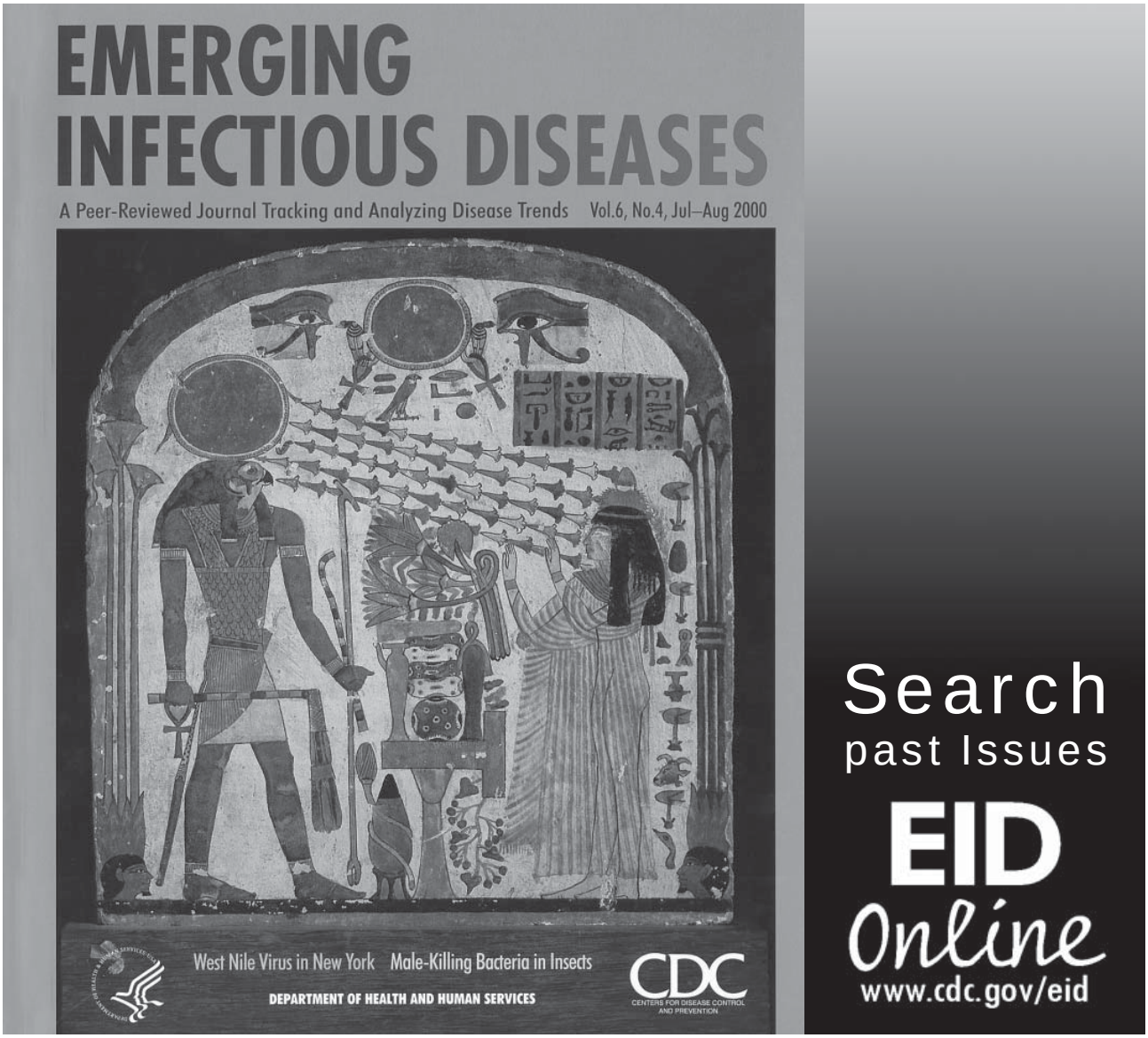

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Geostatistical evaluation of integrated marsh management impact on mosquito vectors using before-after-control-impact (BACI) design

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Abstract

Background: In many parts of the world, salt marshes play a key ecological role as the interface between the marine and the terrestrial environments. Salt marshes are also exceedingly important for public health as larval habitat for mosquitoes that are vectors of disease and significant biting pests. Although grid ditching and pesticides have been effective in salt marsh mosquito control, marsh degradation and other environmental considerations compel a different approach. Targeted habitat modification and biological control methods known as Open Marsh Water Management (OMWM) had been proposed as a viable alternative to marsh-wide physical alterations and chemical control. However, traditional larval sampling techniques may not adequately assess the impacts of marsh management on mosquito larvae. To assess the effectiveness of integrated OMWM and marsh restoration techniques for mosquito control, we analyzed the results of a 5-year OMWM/marsh restoration project to determine changes in mosquito larval production using GIS and geostatistical methods.

Methods: The following parameters were evaluated using "Before-After-Control-Impact" (BACI) design: frequency and geographic extent of larval production, intensity of larval production, changes in larval habitat, and number of larvicide applications. The analyses were performed using Moran's I, Getis-Ord, and Spatial Scan statistics on aggregated before and after data as well as data collected over time. This allowed comparison of control and treatment areas to identify changes attributable to the OMWM/marsh restoration modifications.

Results: The frequency of finding mosquito larvae in the treatment areas was reduced by 70% resulting in a loss of spatial larval clusters compared to those found in the control areas. This effect was observed directly following OMWM treatment and remained significant throughout the study period. The greatly reduced frequency of finding larvae in the treatment areas led to a significant decrease (~44%) in the number of times when the larviciding threshold was reached. This reduction, in turn, resulted in a significant decrease (~74%) in the number of larvicide applications in the treatment areas post-project. The remaining larval habitat in the treatment areas had a different geographic distribution and was largely confined to the restored marsh surface (i.e. filled-

in mosquito ditches); however only ~21% of the restored marsh surface supported mosquito production.

Conclusion: The geostatistical analysis showed that OMWM demonstrated considerable potential for effective mosquito control and compatibility with other natural resource management goals such as restoration, wildlife habitat enhancement, and invasive species abatement. GPS and GIS tools are invaluable for large scale project design, data collection, and data analysis, with geostatistical methods serving as an alternative or a supplement to the conventional inference statistics in evaluating the project outcome.

Background

The salt marsh is a globally important ecosystem in high to middle latitudes along the coastline [1]. Ecologically, salt marshes provide a nutrient rich interface between terrestrial and marine environments utilized by a great variety of animal species. The salt marsh habitat is also of a significant public health importance due to mosquito vector species that have adapted to this harsh environment. Pathogens transmitted by salt marsh mosquitoes include the malaria parasite vectored by *Anopheles atroparvus*, *An. sacharovi*, and *An. labranchiae* in Europe and the Middle East [2], Venezuelan Equine Encephalitis virus vectored by *Aedes sollicitans* and *Ae. taeniorhynchus* in the Americas [3], California group encephalitis viruses vectored by *Ae. dorsalis*, *Ae. caspius*, and *Ae. melanimon* in Europe and western North America [4], and Ross River virus vectored by *Ae. camptorhynchus* and *Ae. vigilax* in Australia [5]. Many of the salt marsh *Aedes* mosquitoes are also important biting pests species in coastal population centers and tourist areas [2,5].

The salt marsh mosquito fauna of Long Island, New York is representative of the Atlantic seaboard of the continental US consisting of 4 species, *Ae. sollicitans*, *Ae. cantator*, *Ae. taeniorhynchus*, and *Cx. salinarius* [6,7]. *Aedes sollicitans* is considered the main epidemic vector of Eastern Equine Encephalitis virus (EEEv) in coastal areas of eastern US [8-10]. The virus has been repeatedly isolated from the wild populations of *Ae. sollicitans* in this region [11,12] and occasionally from *Ae. cantator* and *Ae. taeniorhynchus* [11,13]. *Culex salinarius* is a potentially important epidemic vector of EEEv [14] with multiple field isolations of the virus [15-17]. This mosquito species is also one of the main vectors involved in West Nile virus (WNV) human transmission [18-20]. In addition to numerous WNV isolations from field collected *Cx. salinarius*, the virus was detected in *Ae. sollicitans* (including specimens collected on Long Island) and occasionally in *Ae. cantator* and *Ae. taeniorhynchus* [21,22]. All three salt marsh *Aedes* species can potentially transmit WNV to humans [23,24], although only *Ae. sollicitans* has been associated with the risk of WNV transmission to humans [25].

The public health importance of the salt marsh mosquitoes in the coastal areas of eastern US was apparent long before the discovery of the mosquito-borne viruses. Massive infestations by *Ae. sollicitans* and *Ae. taeniorhynchus* led to the establishment of most, if not all, coastal mosquito control districts [26,27]. Abatement of larval mosquitoes on the salt marsh employed both chemical and habitat modification techniques including ditching of the marsh surface to allow rapid draining of small pools harboring mosquito larvae. Similarly to many coastal areas, Long Island salt marshes were grid ditched in the late 1930s [28]. Although reasonably effective as a mosquito control tool, universal grid ditching was perceived as unnecessary in those parts of salt marshes not producing mosquitoes, and unsatisfactory from ecological and resource conservation perspectives [29]. These concerns resulted in the development of an Open Marsh Water Management (OMWM) technique with the dual goal of non-chemical mosquito control and marsh conservation [29-31]. OMWM targets specific areas of known mosquito larval habitat by employing tidal channels, ponds, and shallow radial ditches to remove these habitats [32]. Tidal channels are designed to improve water circulation and to restore natural tidal regime, while small ponds are created to replace clusters of depressions where mosquito larvae proliferate. Shallow radials connect ponds with scattered depressions to allow access by larvivorous fish. With some modifications, OMWM has been put into practice in many mosquito producing salt marshes along the US Atlantic coast from Massachusetts to Florida, California, and Australia (reviewed in [33]). Invariably, satisfactory results were reported, with the initial estimates suggesting elimination of 40–60 billion mosquitoes annually for every 1,000 OMWM acres [30]. Direct measurements of mosquito production, although infrequent, documented significant reductions (> 85%) in larval or adult mosquito levels (reviewed in [33,29]); however, many studies relied on rather qualitative observations such as number of complaints to assess the outcome. A recent OMWM survey for US Fish and Wildlife Service (USFWS) [34] utilized before-after-control-impact (BACI) design [35] allowing statistically rigorous analysis of the technique's effects [36]. Although we adopted a similar BACI approach for

our project, the two studies differed significantly in scope, goals, and methodology.

Suffolk County occupies most of Long Island, New York and employs both pesticides and water management to control salt marsh mosquitoes. To investigate alternative approaches in compliance with the pesticide usage reduction goal set by the County government, a partnership with USFWS was initiated for a pilot integrated marsh management project at Wertheim National Wildlife Refuge (Wertheim NWR). The following goals of the project included both marsh restoration and mosquito control components: 1) to decrease mosquito production and, by extension, pesticide usage by utilizing OMWM approach, 2) to reduce the vigor and extent of the invasive reed *Phragmites australis* [37], 3) to naturalize marsh surface by eliminating grid ditching, and 4) to maintain or enhance fish and wildlife habitat. This article describes the planning and implementation of the project and focuses on quantitative outcome evaluation for mosquito control. Geographic information systems (GIS) and GPS technology were systematically employed at each stage of the project allowing efficient use of limited resources and enabling development of novel methodology for assessing the project impacts.

Methods

Study area

The project site was located in the ~1,033 ha Wertheim NWR administered by USFWS (Figure 1). Tidal wetlands of Wertheim NWR (~262 ha) and the adjacent areas (~103 ha) form the largest continuous salt marsh on Long Island. The Wertheim NWR salt marshes are comprised of "low" marsh of *Spartina alterniflora* where daily tidal flooding occurs, and "high" marsh with intermittent flooding during storms or high tides occupied by three major plant species – *S. patens*, low-form *S. alterniflora*, and the invasive *Phragmites australis*. These relatively undisturbed and protected areas are surrounded by heavily suburban environment. About 105,000 people (Census 2000) live within 8 km from Wertheim NWR's tidal wetlands, at a typical distance covered by the migratory flight of 50% of newly emerged salt marsh *Aedes* [26]. Thus, public health and quality of life issues associated with salt marsh mosquito species necessitate an active larval control program at Wertheim NWR and adult control program in most of the surrounding communities.

Project planning

The conceptual framework of the project was designed to meet the needs of both vector control and natural resources conservation. OMWM was proposed as a sustainable solution for mosquito control, with a potential to enhance fish and wildlife habitat. The two major features of the OMWM component were ponds and their shallow connectors to tidal channels, which were designed to improve pond water quality and to facilitate access by marine species. Marsh surface restoration and invasive species control were aimed at improving the biological functions of the salt marshes. Eliminating grid ditching was the key step in marsh restoration, while new or enhanced tidal channels/creeks were intended to provide better tidal flow in the *P. australis* infested portions of the marshes. In accordance with the paired BACI design of the study, two treatment and two control areas of the marsh were delineated on-screen in ArcGIS environment using aerial ortho-photographs with creeks and ditches served as natural boundaries. Area 1 (16.0 ha) and Area 2 (18.9 ha) were designated as the treatment areas, whereas Area 3 (10.7 ha) and Area 4 (18.5 ha) were designated as the control areas (Figure 2). For preliminary surveillance, loci of mosquito breeding and vegetation cover were georeferenced by GPS hand-held devices and used to create the base map. New hydrologic features (i.e. ponds, tidal channels, and connectors) in the treatment areas were plotted on-screen in ArcGIS environment using the base map. A small number of mosquito ditches suitable for hydrologic connectivity were retained for naturalization; most grid-ditches were targeted for filling in. Following the delineation of the areas and the proposed alterations, all the required permits were obtained from the local, state, and federal agencies.

Project implementation

To assist in mapping out the proposed hydrologic features on the ground, a network of georeferenced points was established in the treatment areas using Trimble® GPS receivers (Trimble Navigation Ltd., Sunnyvale, CA). The outline of ponds, tidal channels, connectors, and larval habitat targeted for elimination by filling in dense concentrations of larvae producing potholes [29] were staked out using the hydrologic feature map and georeferenced points as a guide. The stake locations were recorded by hand-held GPS receivers and the data were visualized in ArcGIS overlaying the hydrologic feature map. The stake positions were then adjusted if necessary. The following alterations were made in Area 1 (March 2005) and in Area 2 (February-March, 2006). Ponds, tidal channels, and connectors were constructed using low-ground pressure (< 2 psi) machinery. The majority of pre-existing mosquito control ditches in the marsh were filled with the material extracted during the pond excavation process using rotary ditchers and excavators. The remaining ditches were naturalized by adding curves and other features commonly found in natural salt marsh channels and creeks. Additional tidal channels were created with the overarching goal of increased tidal circulation into the interior of the salt marshes. The excess pond/tidal creek excavated material was used to grade hummocky high

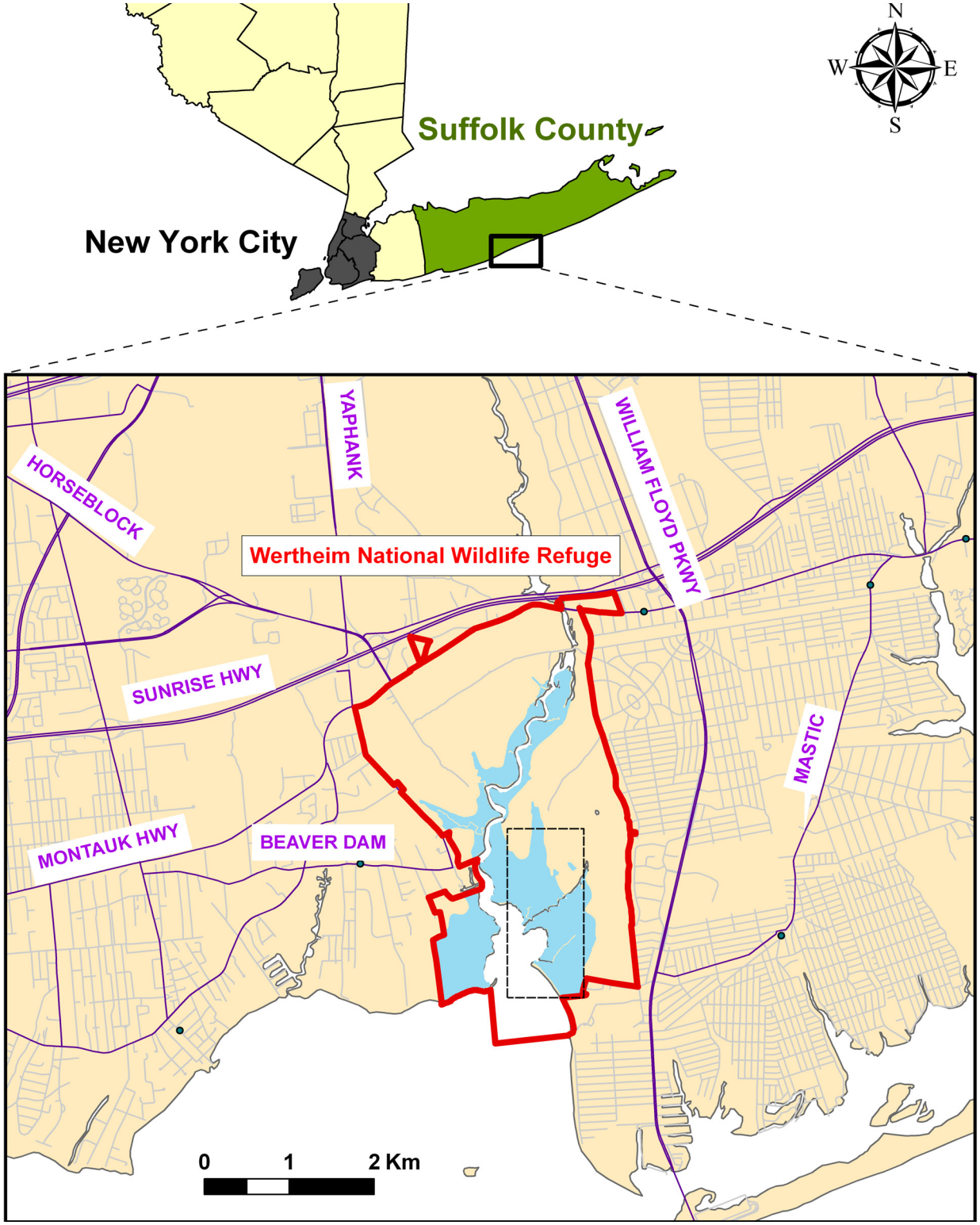

Figure 1
The Wertheim project study area. Red line shows Wertheim NWR boundary; dashed line indicates approximate geographic extent of the study area. Tidal wetlands are indicated in blue. Major roads (purple) and residential streets (gray) are also shown.

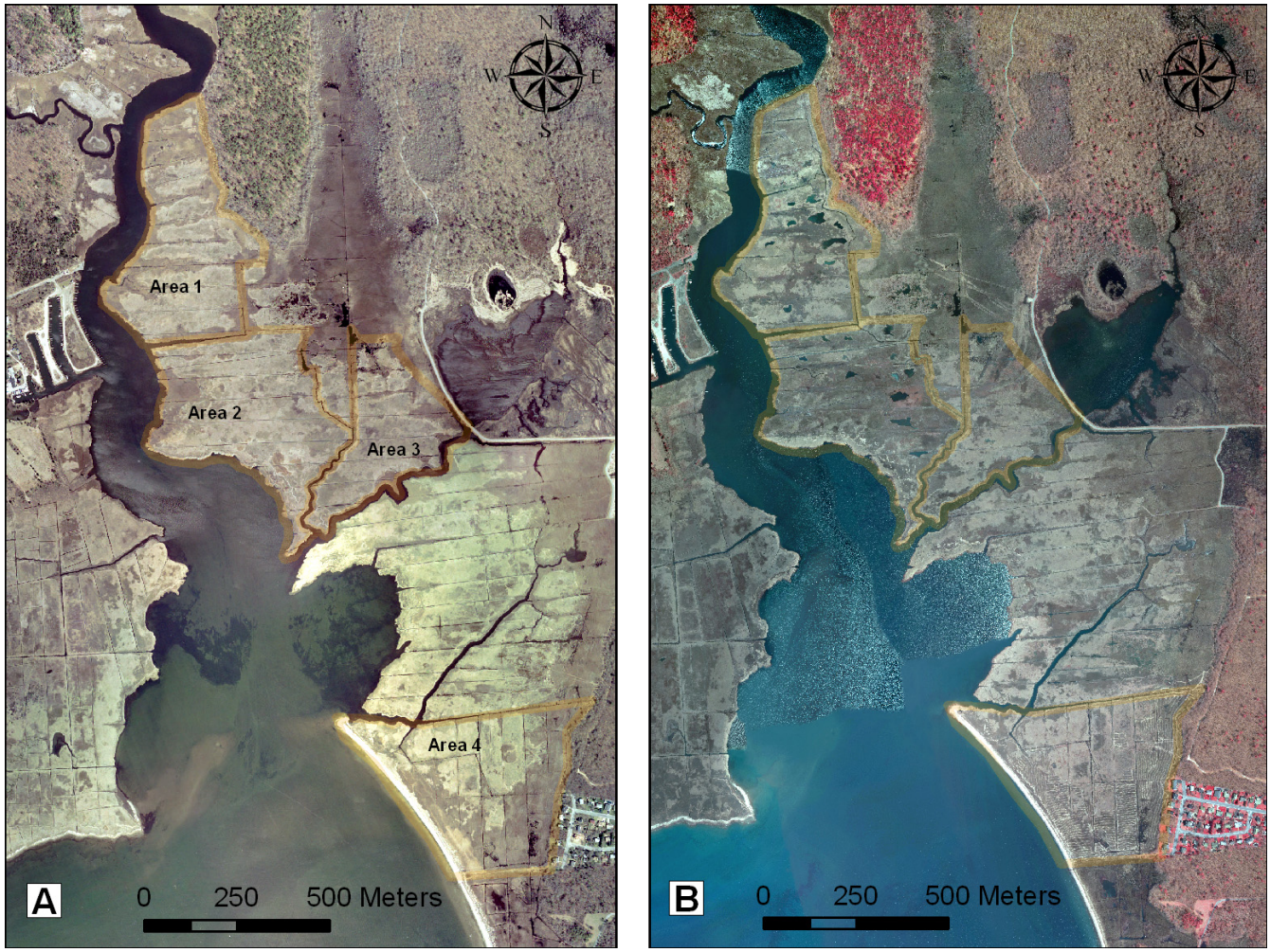

Figure 2
Aerial view of the study areas in 2004 (A, pre-project) and 2007 (B, post-project). Treatment: Areas 1 and 2, Control: Areas 3 and 4.

marsh terrain where larval habitat had been found by the preliminary surveillance (i.e. "backblading").

Entomological data collection

Two sampling procedures were employed to collect larval data. A systematic sampling protocol [34] was carried out to establish random transects (n = 5 in Areas 1 and 2 each, n = 4 in Areas 3 and 4 each) with sampling stations distributed equally at every 40 meters with sampling taking place at and between the stations (i.e. every 20 meters) resulting in a total of 24 sampling points in each Areas 1 and 2, and a total of 20 sampling points in each Areas 3 and 4. These transects extended from the upland portion of the salt marsh seaward into the low marsh. A second procedure utilized targeted sampling, a more common method used by mosquito control professionals, whereby suitable larval habitats, i.e. pools of standing water, were searched for mosquito larvae across all 4 Areas. These surveys intended to be comprehensive, so the majority of potential larval habitats within an area were sampled each time. At least 25 samples were obtained per area per visit unless the marsh surface was either dry (no standing water) or completely flooded.

Each area was visited weekly for targeted sampling from early May to mid September, a period corresponding to the active mosquito season in this region. Additionally, transect sampling was carried out monthly 4 to 7 days following a high tide inundation to maximize the chances of finding mosquito larvae on the salt marsh [34]. Mosquito larvae were collected using standard mosquito dippers (Bioquip Inc., Rancho Dominguez, CA). Custom database design software for Windows CE (Visual CE, Syware, Cambridge, MA) was developed to run on a hand-held

personal digital assistant (PDA; Dell Axim™ X51, Dell Inc., Round Rock, TX) coupled with a GPS device (GPSlim 236, Holux Technology Inc., Hsinchu, Taiwan). The program recorded the following data: geographic coordinates, time, number of mosquito larvae and pupae, type of habitat, sampling method with transect/station information, and comments. The data were uploaded directly into a MS Access database using Microsoft ActiveSync. For data quality assurance, the sampling points were visualized in ArcMap and any errors were identified and corrected.

As a rule, 1 to 5 typical larval samples from each area were brought to laboratory for microscopic species identification using morphological mosquito keys [38]. First instar larvae were allowed to progress to later instars, and larvae of *Culex* spp. were allowed to emerge as adults to confirm positive identification. Adult *Cx. salinarius* were separated from those of *Cx. pipiens* by using morphological characters [38] as well as molecular techniques [39].

Entomological data evaluation and statistical analysis

Three parameters were evaluated to assess the outcome of the project: frequency and geographic extent of larval production, intensity of larval production, and the overall impact of the project (both OMWM and restoration components) on mosquito larval habitat. In accordance with BACI design, the analyses were performed on aggregated "Before and After" data and also followed through time to compare "Control" and "Impact" (i.e. treatment) areas to identify changes attributable to the intervention.

Presence or absence of mosquito larvae, a dichotomous variable, was used to evaluate geographic patterns of larval production. Each sample was thus classified as either 1 (= positive dip) or 0 (= negative dip). Global and Anselin Local Moran's I were calculated to assess the overall spatial patterns and local geographic clusters of positive or negative dips using ArcMap 9.3 software (ESRI Inc, Redlands, CA). Normalized Z-scores, or the number of standard deviations, were interpreted to represent clusters (Z-score > 2.0), outliers (Z-score < -2.0), or random distribution (-2.0 < Z-score < 2.0) at statistical significance $P < 0.05$. To visualize a statistical surface of mosquito larval production over the entire study area, Voroni tessellation was performed to create Thiessen polygons around each data point classified by presence or absence of mosquito larvae and the Z-score. Adjacent polygons in the same category were dissolved and smoothed to produce the final map.

To define statistical significant clusters of positive and negative dips spatially and temporarily, a spatial scan cluster analysis was carried out using free SaTScan™ software [40]. Positive dips (the case file) and negative dips (the control file) were analyzed by space-time statistic with Bernoulli probability model for dichotomous data employing the following settings: year as the time period, maximum non-overlapping spatial cluster size of 0.5 km radius roughly corresponding to the extent of each treatment and control area, scan for high and low values, and 4-year temporal window with pure spatial clusters (i.e. present each year). The statistical significance was calculated by Monte-Carlo simulation with 9999 replications.

To determine the intensity of mosquito larval production (termed "breeding intensity"), an ordinal scale was developed based on field observations. Dips with 1 to 2 larvae per dip were classified as "low" (rank = 1), those with 3–5 larvae per dip as "intermediate" (rank = 2), and those with > 5 larvae per dip as "high" (rank = 3). Only positive dips were included in the analysis using global (General G) and local (Gi*) Getis-Ord statistic in ArcMap 9.3 to assess the overall distribution of breeding intensity values and to identify geographic locations with elevated breeding intensity, i.e. non-random clusters. Similarly to Moran's I statistic, normalized Z-scores were interpreted to represent clusters (Z-score > 2.0), outliers (Z-score < -2.0), or random distribution (-2.0 < Z-score < 2.0) at statistical significance $P < 0.05$. To visualize distribution of positive dips on the marsh surface, kernel density was calculated using 5 × 5 meter grid and 50 meter search radius.

Post-project problem area characterization

ArcMap 9.3 buffering tool and sampling tools available with Hawth's ArcMap extension [41] were utilized to evaluate changes in mosquito larval habitat before and after the treatment. Specifically, the impact of filling in mosquito ditches (a marsh restoration technique) on larval habitat was assessed by determining proportion of positive dips at close (< 5 meters), medium (5–15 meters), or long (> 15 meters) distance range. All non-spatial statistical analyses were conducted in SPSS v. 15.0 (SPSS Inc, Chicago, IL) software and assumed statistical significance at $P < 0.05$.

Mosquito abatement

Mosquito abatement measures consisted of aerial application of larvicides by helicopter. Routine mosquito control program continued throughout the study period using the standard criteria set by the state regulatory agencies and USFWS. At least 25 samples were required from each area to meet the minimum larviciding threshold of 0.2 larvae per dip. Other considerations included the extent of the infested area (i.e. total number of positive dips), weather, and environmental conditions directly affecting mosquito larval habitat such as marsh flooding. These criteria were uniformly applied without regard to the status (impact or control) of the particular location to determine whether a larvicide application was justified. Two types of larvicides were used in the control program. Vectobac 12AS (*Bacillus*

thuringiensis var. *israelensis*; ValentBioscience Corp.) is a liquid bacterial product applied when early larval instars (stages 1–2) were detected. Altosid Liquid Larvicide Concentrate (methoprene; Central Life Science/Wellmark™) is an insect growth regulator applied against late instar larvae (stages 3–4). When both early and late larval instars were present simultaneously, a combination of Vectobac and Altosid was used.

Before and after treatment effects on number of larvicide applications and proportion of time the larviciding threshold was reached were analyzed according to the published guidelines for BACI designs [42]. The differences between control and treatment sites were computed and compared before and after treatment by Mann-Whitney test. SPSS v. 15.0 was used for data processing and statistical analyses.

Results

Project implementation

Twenty three ponds with the approximate total surface area of 1.1 ha were constructed in the treatment Areas 1 and 2 (Figures 2B and 3). The ponds were constructed with a "teaspoon" profile with at least one deeper sump approximately 0.5–0.75 m deep to serve as a fish refuge. The gradually sloping sides of the ponds were intended to allow fish access into the marsh during flooding, and to serve as foraging habitat for shorebirds. In Area 1, 11 ponds with the size range of approximately 191–1678 sq. meters (mean = 556 sq. meters), and the total area of 0.61 ha were constructed and the material used to completely fill 9 out of 11 existing mosquito grid ditches. The remaining 2 ditches were naturalized and incorporated into a tidal creek system with a new tidal channel reaching into the upland larval habitat and areas heavily infested by *P. australis*. In Area 2, 12 ponds with the size range of approximately 181–887 sq. meters (mean = 432 sq. meters), and the total area of 0.52 ha were constructed and the material used to fill completely or partially 10 out of 11 existing mosquito grid ditches. The ponds constituted about 3% of the total surface area in the treatment marshes. Assuming 1 m as an average width of a filled ditch, the net gain of the open water habitat due to pond construction within the treatment areas was about 0.6.ha or about 1.7% of the total area. The remaining ditch and ditch spurs were naturalized and incorporated into a tidal system following what appeared to be remnants of pre-ditching channels (Figure 3). The pre-existing channel bordering the east side of the area was extended. No alterations were carried out in the control area (Areas 3 and 4), which represented a mix of low and high marsh with significant *P. australis* presence and intact grid ditching system. All four areas pre- and post-treatment are shown in Figures 2 (aerial imagery) and 3 (treatment areas post-project).

Entomological data

A total of 12,946 samples (dips) were collected in the study area between 2004 and 2008. The sampling effort was fairly uniform (Table 1). Some of the samples were "dry", i.e. no standing water was present at the sampling station (transects), or at the previously identified mosquito larval habitat (targeted), usually during the periods when tidal or rain surface water was completely drained from the salt marsh. Approximately 49% of the transect samples were dry, significantly higher than ~13% of the targeted samples (Chi-square test, $X^2 = 1859.9$, df = 2, $P < 0.001$; Table 2). Only about 10% of transect samples with water contained mosquito larvae, which was significantly lower than ~27% in targeted sampling (Chi-square test, $X^2 = 211.4$, df = 1, $P < 0.001$; Table 3). Overall, only ~7% of all positive dips came from transect sampling.

Immature stages of 3 mosquito species, *Ae. sollicitans*, *Ae. cantator*, and *Cx. salinarius* were collected during the study period. The presence of *Cx. salinarius* in the upper salt marsh was unexpected; thus, the close association of this species with the salt marsh habitat was investigated and characterized [7]. Although *Ae. sollicitans* was the most commonly found species in all 4 areas throughout the season, *Cx. salinarius* sometimes predominated in Area 3 when more permanent brackish water from rain events accumulated on the marsh during this species' peak season from late July through early September.

Entomological data evaluation and statistical analysis

The proportion of positive dips (i.e. those containing larvae, mean% ± SE) was reduced from 28.8% ± 4.1 to about 7.5% ± 1.1 in the treatment areas (Areas 1 and 2; Figure 4) post-project, while remaining similar (31.9% ± 3.6 versus 29.2% ± 2.5) in the control areas (Areas 3 and 4; Figure 4) post-project. The spatial characterization of the larval production over the entire study area before and after project implementation is shown in Figure 5A. The overall data pattern was highly clustered and auto correlated both before (Global Moran's I = 0.17, Z-score = 29.4, $P < 0.01$) and after (Global Moran's I = 0.20, Z-score = 101.8, $P < 0.01$) the impact. Anselin Local Moran's I identified statistically significant clusters of larval production in all 4 areas prior to the intervention. These clusters were completely eliminated from Area 1 and significantly reduced in Area 2 following the project implementation in these two treatment areas. Conversely, significant clusters of larval production present in the control areas (Areas 3 and 4) pre-project remained in place post-project resulting in highly contrasting pattern between the treatment (absence of larvae clustering) and control (presence of larvae clustering surrounded by negative outliers) areas. Overall, the breeding intensity, i.e. the average number of larvae found in positive dips, remained similar in both treatment and control areas (Figure 4) post-project. How-

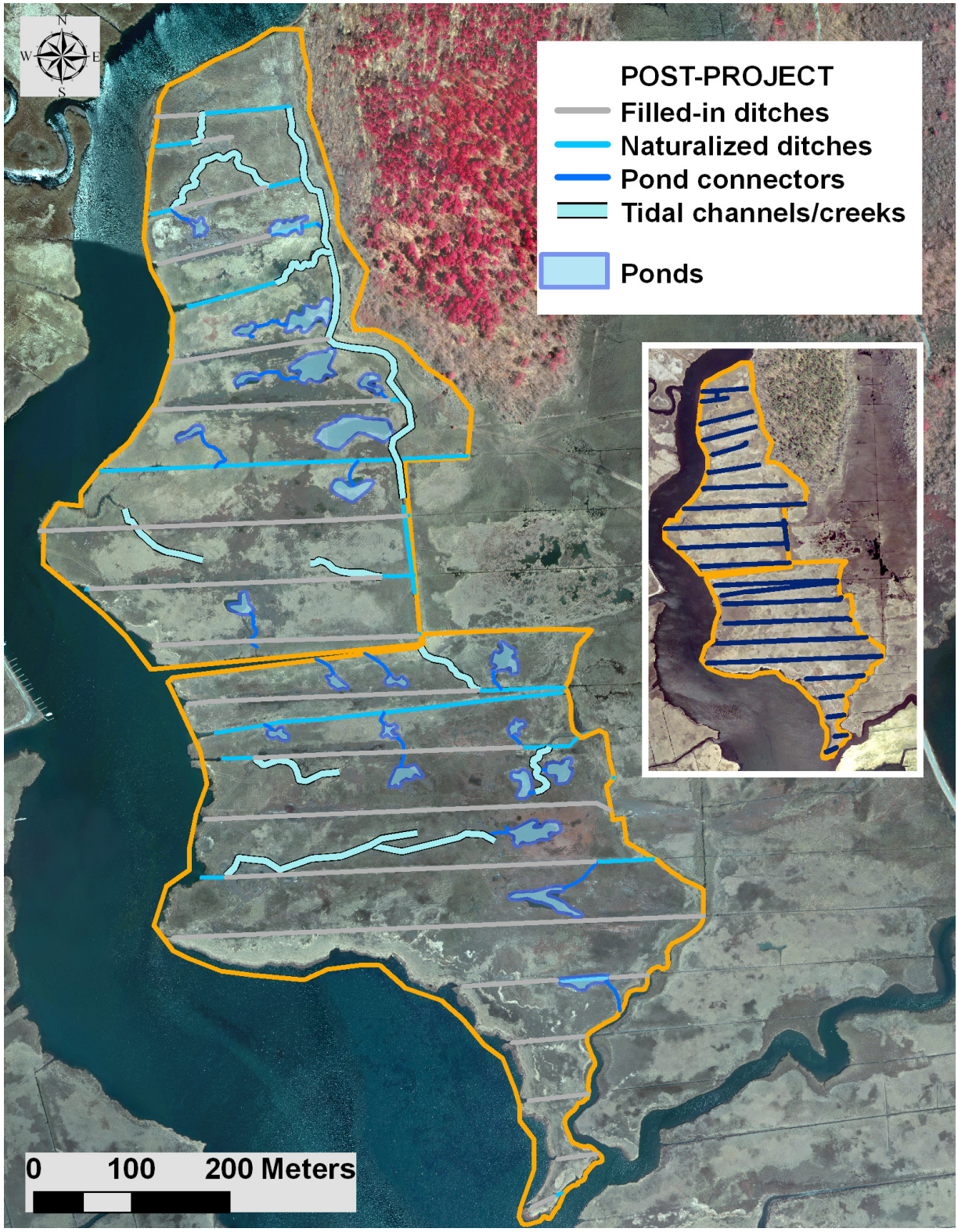

Figure 3
Salt marsh alterations in Areas 1 and 2 (i.e. treatment) post-project. Insert shows grid-ditching system (blue lines) pre-project.

Table 1: Number of samples (dips) taken per area per year.

Year	Area 1	Area 2	Area 3	Area 4	Total
2004	658	588	360	418	2024
2005	353	458	339	497	1647
2006	644	950	729	1022	3345
2007	856	823	621	855	3155
2008	723	658	677	717	2775
Total	3234	3477	2726	3509	12946

ever, spatial analysis showed locations of elevated breeding intensity clustered both before and after the intervention (Getis-Ord General G = 0, Z-score = 3.8, $P < 0.01$), but statistically significant clusters were found exclusively in the control area (Areas 3 and 4) following the intervention demarcating focal points of elevated larval production (Figure 5B).

The steep reduction in the proportion of positive dips occurred immediately after the intervention in both Area 1 (year 2005) and Area 2 (year 2006), whereas no such changes were observed in Areas 3 and 4 (Figure 6). This reduction in larval production remained consistent through 2008. Similarly, the drastic changes in the spatial patterns of larval production within the treatment areas occurred immediately following the intervention and remained consistent through 2008 (Figure 7). Although the overall larval distribution remained highly clustered each year ($P < 0.01$), statistically significant larval clusters in Area 1 were greatly reduced in 2005 (1st post-project season) and eliminated by 2006. Similarly, statistically significant larval clusters in Area 2 were greatly reduced in 2006 (1st post-project season); some residual mosquito breeding occurred on some of the restored marsh surface on top of the filled-in mosquito ditches (see the next section). Unlike the treatment areas, the control areas (Areas 3 and 4) exhibited remarkable consistency, with statistically significant clusters of larvae found in the same geographic locations each year despite discernible inter-annual variability (e.g. "low clustering year" = 2005 and "high clustering year" = 2006). The breeding intensity (based on positive dips only) remained similar in both treatment and control areas through 2008 (Figure 6). Spatial analysis indicated clustering of the locations with elevated breeding intensity in all years ($P < 0.01$) but 2007 ($P = 0.1$). Statistically significant clusters were generated sporadically and occurred in Area 2 (pre-project) and Area 3 in 2005, and were limited to the control areas only post-project (Figure 7).

Table 2: Proportion of samples (dips) containing water in transect versus targeted sampling. N/R – not recorded.

Samples (Dips)		Water			Total
		Yes	No	N/R	
Transect	Count	1646	1590	35	3271
	%	*50.3*	*48.6*	*1.1*	
Targeted	Count	8152	1227	296	9675
	%	*84.3*	*12.7*	*3.1*	

Table 3: Proportion of positive samples (dips containing mosquito larvae) per samples with water in transect versus targeted sampling.

Samples (Dips)		Larvae		Total
		Yes	No	
Transect	Count	172	1474	1646
	%	*10.4*	*89.6*	
Targeted	Count	2233	5919	8152
	%	*27.4*	*72.6*	

To assess statistical significance of patterns of larval production both spatially and temporally, a space-time scan analysis was performed (Figure 8). Most of the treatment areas (Areas 1 and 2) were contained within 2 statistically significant ($P < 0.001$) clusters of low, i.e. "no larvae found" values. These low clusters became significant immediately following the intervention (2005 for Area 1 and 2006 for Area 2) and remained so until 2008. The control areas (Areas 3 and 4) contained 2 statistically significant ($P < 0.001$) clusters of high, i.e. "larvae present" values. Area 3's high cluster was present throughout the study period (2004–08); Area 4's high cluster was not statistically significant in 2008.

Characterization of the post-project residual larval habitat in the treatment areas

Despite significant reduction in the frequency of positive dips within the treatment areas (Areas 1 and 2), some residual breeding continued post-project. Field personnel noted that a considerable proportion of post-project larvae collections were made from the filled-in mosquito ditches, especially in Area 2 (Figure 9). In some ditches, the topsoil used as a fill settled down thereby forming a slightly concave surface, which held standing water suitable for mosquito larvae. Statistical analysis confirmed the role of the filled-in ditches as larval habitat. Pre-intervention, before the ditches were filled to restore the marsh surface in the treatment areas, only a minute proportion (~2%) of positive dips were found in close vicinity (i.e. within 5 meters) of these ditches compared to about one-quarter (~27%) of the total after being filled in (Table 4). Only ~17% of the positive samples were collected within 15 meters from these ditches pre-project compared to ~60% post-project. These differences were statistically significant (Chi-square test, $X^2 = 182.6$, df = 2, $P < 0.001$) and corroborated the hypothesis that filled in ditches represented the main residual larval habitat for mosquitoes

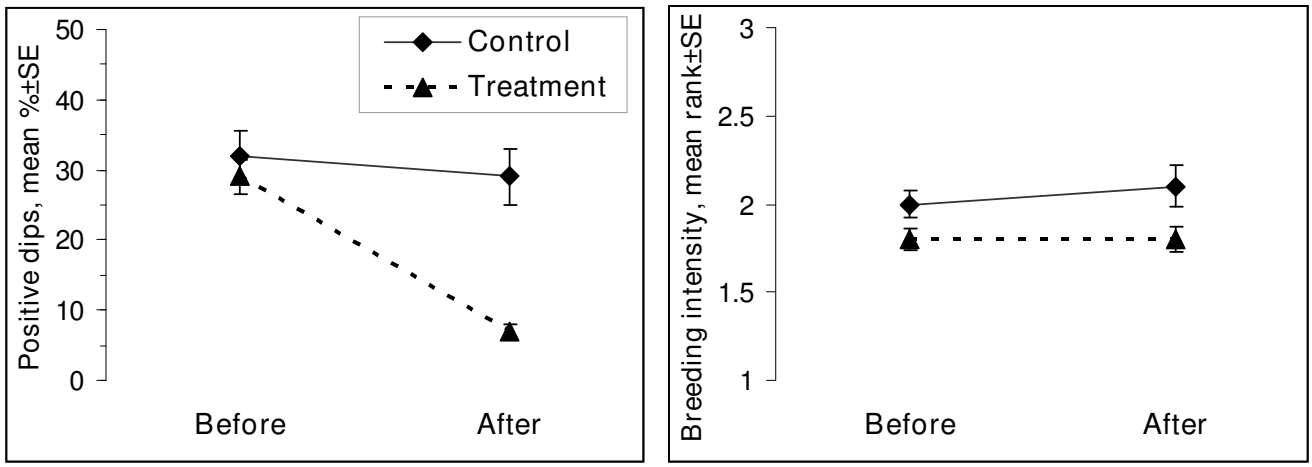

Figure 4
Weekly mean percent of positive dips ± SE containing larvae and weekly mean breeding intensity, rank ± SE (1 = low, 2 = intermediate, 3 = high; positive dips only) in treatment (Areas 1 and 2) and control (Areas 3 and 4) areas before and after treatment.

post-project. However, only a small proportion of the restored marsh surface supported larval habitat. Mosquito larvae were mostly found near 3 out of 19 filled-in ditches and limited to ~835 linear meters (21.6%) out of ~3,867 total filled-in linear meters.

Inspection of the post-project maps showing the distribution of larvae in the treatment areas (2006 to 2008) indicated a trend of diminishing residual larval habitat. This was also supported by the decreased likelihood of clustering in Area 2, where most of the residual breeding occurred (Figure 9). On the ground, many sections of the filled-in ditches that had been bare and concave with ample larval habitat in 2006–07 were rapidly re-vegetating and becoming level with the marsh surface in 2008. Based on these observations, the process of reduction in the mosquito habitat on or near filled-in ditches is expected to continue in the future.

In addition to the filled-in ditches, two problems of a lesser magnitude contributing to residual mosquito breeding were identified: a) insufficient connectivity with some of the newly created ponds and tidal channels, potentially leading to limited access of the affected areas by larvivorous killifish, and b) increased water accumulation due to a plugged ditch. However, these problems were minor and could be easily corrected without significant impact on the salt marsh surface by either creating shallow radials or removing the plug.

Mosquito abatement

The mean number of larvicide applications per month during the mosquito season (May–September) for each area is shown in Table 5 for 4 years preceding the project (2001–04) and 4 years post-project (2005–08). The pre-project period was extended by 3 years for this analysis to increase the statistical power and to minimize random trends.

The number of larvicide applications per month (mean ± SE) in the treatment areas was reduced by approximately 74% from 1.95 ± 0.20 pre-treatment to 0.51 ± 0.10 post-treatment. The number of applications (mean ± SE) in the control areas remained similar, 2.08 ± 0.20 pre-treatment to 1.80 ± 0.19 post-treatment. The treatment-control difference was significantly higher post-project (Mann-Whitney U = 61.0, $P < 0.001$) indicating a statistically significant reduction in the number of larvicide applications (Figures 10A and 11). The treatment and control areas pre-project similarities and post-project differences were consistent throughout the study period (Figure 11).

To determine whether the observed reduction in the average number of monthly larvicide applications was due to reduced frequency of positive dips as suggested by the spatial data analysis, the average proportion of weeks per month when the larviciding threshold (> 0.2 larvae/dip) had been reached was computed for each area and compared before (2004–05) and after (2005–08) treatment (Figure 10B). To increase the sample size, Area 2 was compared to Areas 3 and 4 to provide pre-treatment data for 2005, while Area 1 was also compared to the two control areas to provide post-treatment data for the same year. The average proportion of weeks (mean ± SE) above the threshold level for Areas 1 and 2 decreased by approximately 44% from 0.57 ± 0.1 pre-treatment to 0.25 ± 0.05

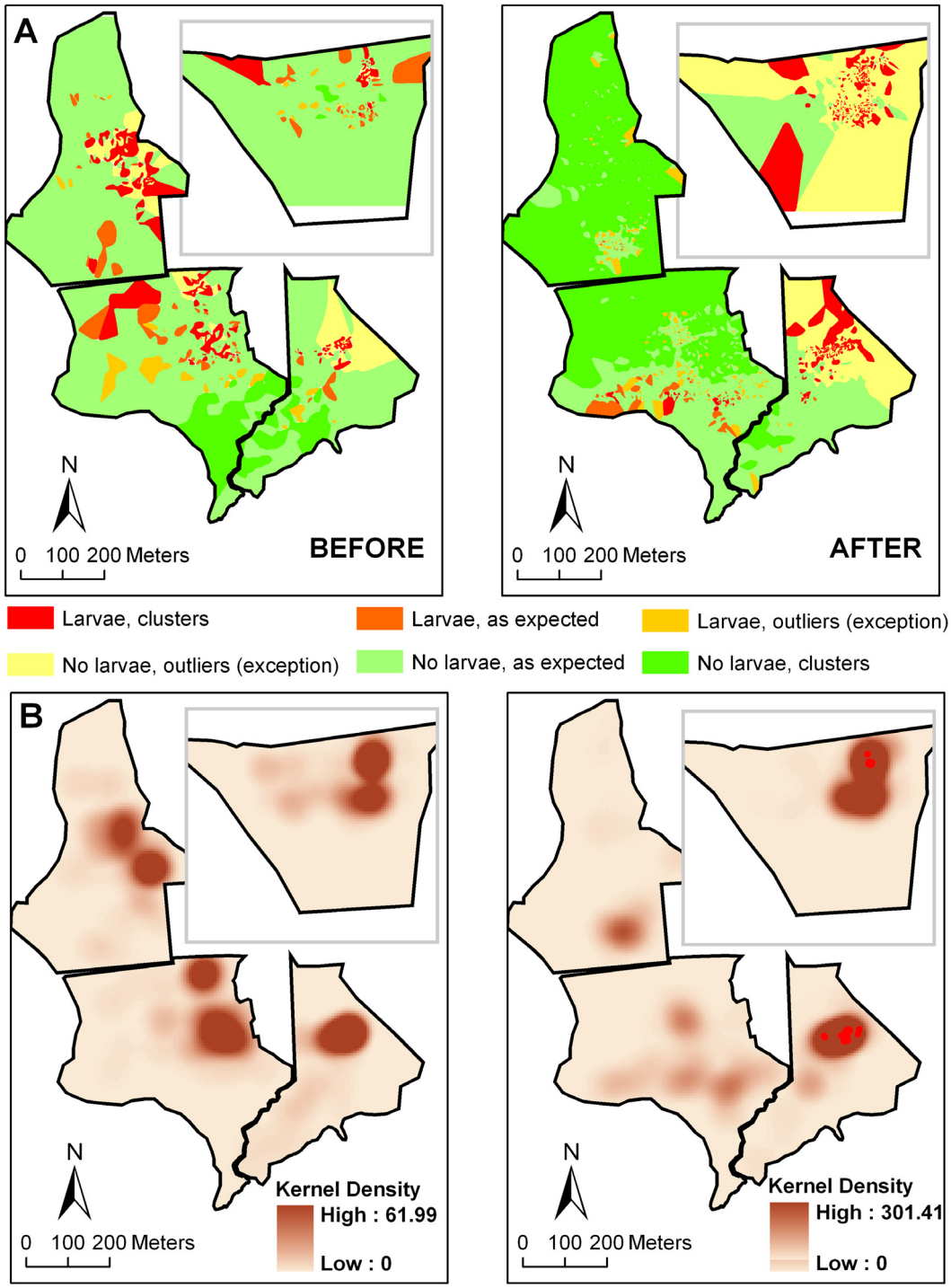

Figure 5

A) Likelihood of finding larvae (from high to low) before and after the project implementation based on Anselin Local Moran's I. B) "Hot spots" of elevated breeding intensity (positive dips only) based on Getis-Ord Gi* (Kernel density based on number of positive dips; Red dots = clusters of high values Z > 2.0). Treatment: Areas 1 and 2, Control: Areas 3 and 4.

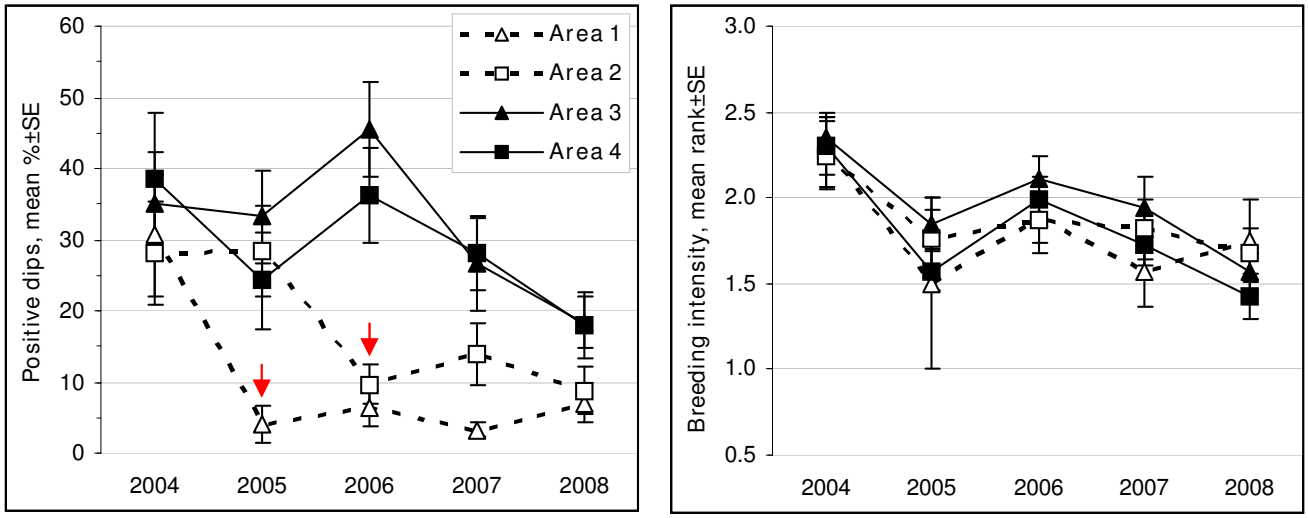

Figure 6
Weekly mean percent of positive dips ± SE containing larvae and weekly mean rank breeding intensity ± SE (1 = low, 2 = intermediate, 3 = high) in positive dips by in treatment (Areas 1 and 2) and control (Areas 3 and 4) areas by year. An arrow indicates the first post- treatment year.

post-treatment, while that for the control areas remained similar, 0.57 ± 0.1 pre-treatment and 0.60 ± 0.05 post-treatment. The difference in the proportion of weeks between the treatment and the control areas was significantly higher post-treatment (Mann-Whitney U = 21.5, P = 0.001) suggesting a statistically significant reduction in the number of weeks when larvicide applications were justified in Areas 1 and 2 following treatment.

Discussion

This study's goals, design, and analysis differed significantly from the previous investigations on Open Marsh Water Management (OMWM). The goals of the Wertheim Integrated Marsh Management (IMM) project were not limited to achieving reduction in mosquito production (the OMWM component), but also concurrently included restoring the marsh surface by eliminating grid-ditching, and controlling the invasive species *P. australis*. To better assess the impact of these techniques on the marsh flora and fauna, a quasi-experimental before-after-control-impact (BACI) study design with 2 pairs of impact and control sites was utilized. A BACI approach was selected because it offered the closest approximation of a field study to a full experimental design to detect and evaluate the impact (i.e. treatment) effects [35,42]. For mosquito production, geostatistical analysis of the spatial pattern of larval distribution on the marsh surface was used as an alternative to conventional statistical methods to improve the validity of the statistical analysis, to better assess the project effectiveness, and to fully understand the underlying causes of some of the challenges encountered during the study.

Initially, we planned to use random sampling at transect locations to evaluate changes in mosquito production by conventional statistical analysis [34] supplemented by geostatistical analysis of targeted sampling traditionally employed by mosquito control districts. However, about one-half of transect samples were dry (= "no data"), significantly higher than about 13% of those for targeted samples. In addition, only about 9 transect samples with larvae compared to about 112 targeted samples were collected on average per Area each year. Due to high degree of spatial dependency or autocorrelation, the amount of information in the few positive transect samples was further reduced leading to smaller effective sample size, underestimated variance, and increased type I error [43]. Thus, a statistical analysis based exclusively on the transect samples would result in a very low statistical power from a purely technical perspective and questionable biological significance. Numerically superior targeted samples, on the other hand, violated two classical inference assumptions, namely independency between samples (similarly to transect samples) and random selection of the sampling locations. To circumvent these two issues commonly encountered in vector control practice, geostatistical approach was used instead. Presence of spatial dependency is one of the central assumptions in geostatistics, and its magnitude is an important parameter for assessing the spatial patterns such as larval clustering.

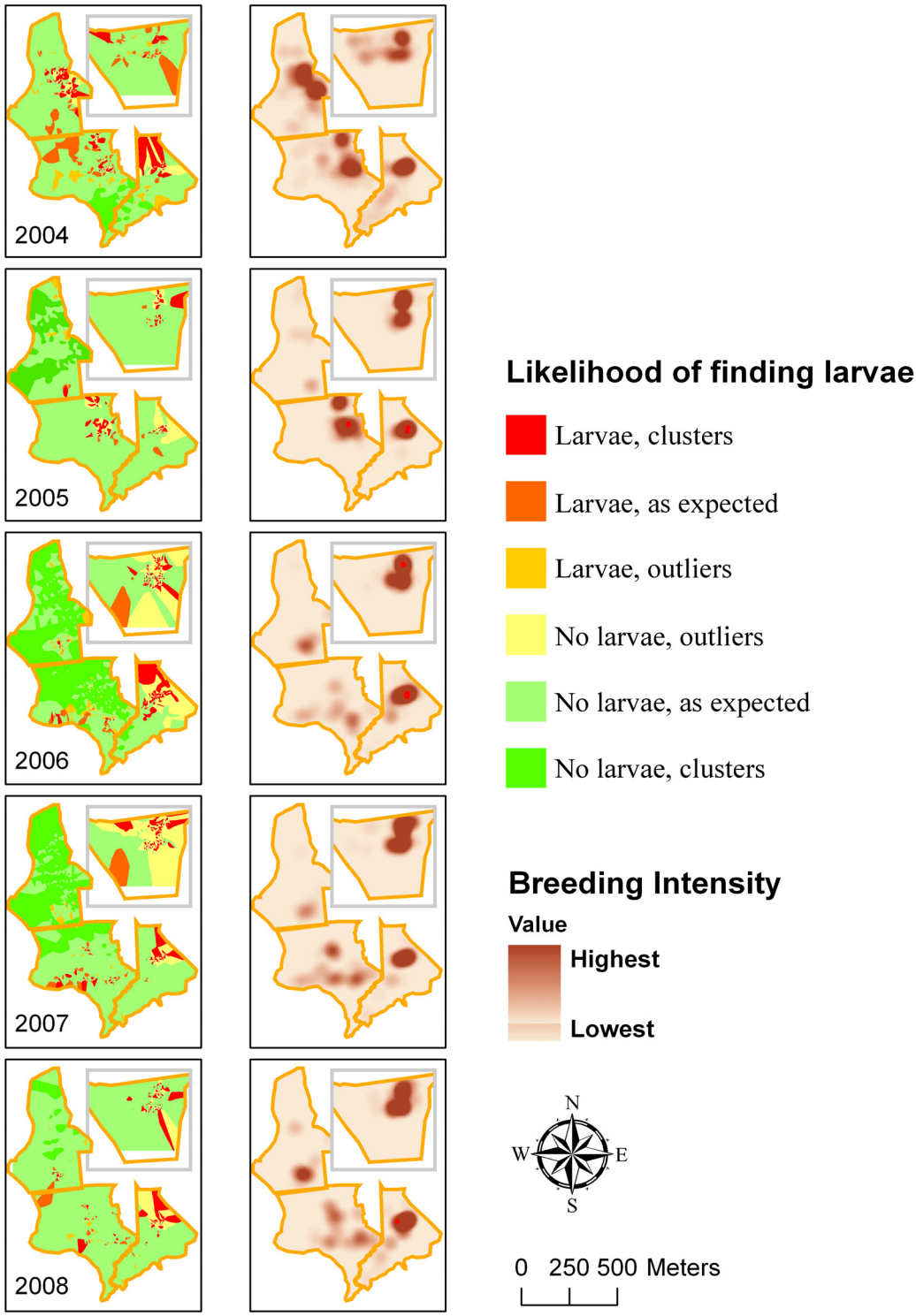

Figure 7
Left Column: Likelihood of finding larvae (from high to low) by year based on Anselin Local Moran's I. Right Column: "Hot spots" of elevated breeding intensity based on Getis-Ord Gi* (Kernel density based on number of positive dips; ● Red dots = clusters of high values Z > 2.0). Treatment: Areas 1 (2005–08) and 2 (2006–08), Control: Areas 3 and 4.

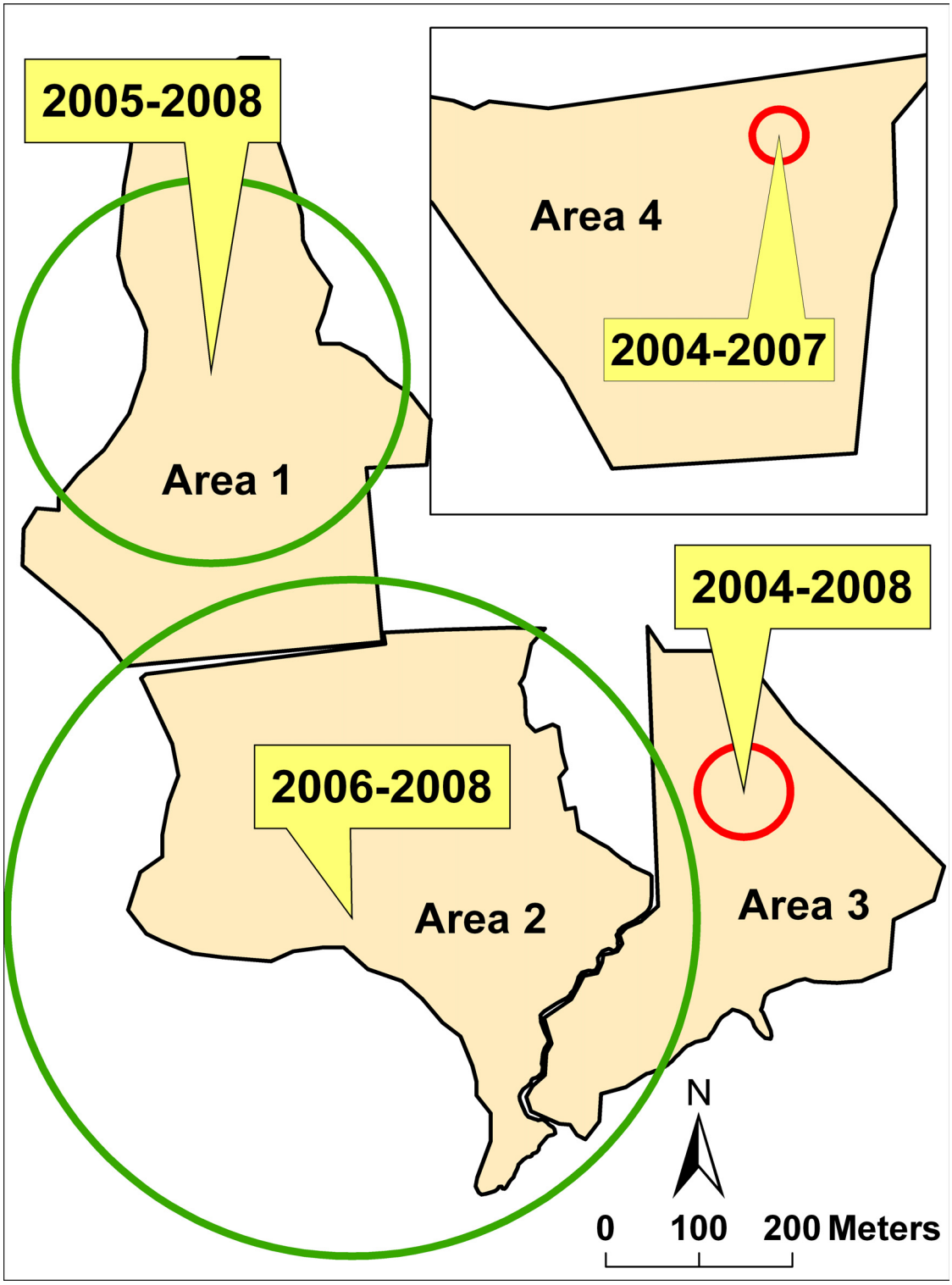

Figure 8
SaTScan™ space-time cluster analysis of larvae presence or absence. Geographic extent of statistically significant ($P < 0.001$) clusters of larval presence is indicated by a red circle; geographic extent of statistically significant ($P < 0.001$) clusters of larval absence is indicated by a green circle. Statistically significant time period, years at $P < 0.001$ is shown in yellow boxes. Treatment: Areas 1 (2005–08) and 2 (2006–08), Control: Areas 3 and 4.

Table 4: Number of positive dips as a function of distance to filled in mosquito ditches before and after the intervention.

Treatment		Distance to filled ditch, m		
		5	5–15	> 15
Before	Count	10	66	371
	%	*2.2*	*14.8*	*83.0*
	Std. Residual	**-6.6**	**-3.7**	**5.4**
After	Count	107	131	161
	%	*26.8*	*32.8*	*40.4*
	Std. Residual	**7.0**	**4.0**	**-5.7**

Standardized residual (significant if > 2.0 or < -2.0, bold letters) is indicated.

Although probability sampling is required for an unbiased estimate of population parameters in conventional statistics, geostatistical model-based approach does not require random selection of sampling location [43]. In addition, the representativeness of targeted sampling design used in this study was enhanced by the spatial scope seeking to encompass the entire population and by replication in time over a 5-year study period.

Commonly, quantitative assessment of larval populations relies on number of mosquito larvae per dip. However, this number is highly variable and dependent on many factors unrelated to true mosquito density. For example, mean number of larvae per dip varied significantly both among different operators and between repeated samples taken by the same operator from the same source [44]. Dipper samples could not differentiate population densities below ~280/m^2, and more than 6,000 samples were required to estimate the population parameters with α = 0.05 and β = 0.1 [45]. Other factors more specific to the salt marsh mosquitoes may include the size of the pools, presence of larval aggregates, and time of the day, among other factors. Thus, Service's [46] extensive review of entomological literature concluded that larvae per dip could not serve as a true estimate of the larval population. Accordingly, we used presence/absence of mosquito larvae as the main mosquito population parameter in this study. From an operational perspective, the location and the geographic extent of larvae producing areas (i.e. "hotspots") are more important for implementing targeted mosquito abatement program. Large areas of salt marsh mosquito larval habitat can be rapidly characterized using presence/absence data entered into a handheld GPS unit while minimizing technical errors and increasing effective utilization of field personnel. Given similar breeding intensity (i.e. average number of larvae in positive dips) between the treatment and the control areas, frequency of positive samples was also directly proportional to the mean number of larvae per dip in this study.

The OMWM concept was originally developed to provide effective long term control of mosquito larvae by source reduction and biological control. Field data on OMWM projects collected over a 40-year period have been largely supportive of this statement. The magnitude of the reduction in mosquito production generally ranged from 85% to complete elimination [29,33]. For example, Ferrigno [47] reported a reduction from 3.7 × 10^6 larvae/acre pre-OMWM to almost zero post-OMWM in the upper marsh *S. patens* treatment areas, while 1.5 × 10^6 larvae/acre were detected on average in the control areas. Similarly, a 99% difference of 3.3 versus 0.02 larvae/dip was found between ditched marsh control and OMWM sites in Massachusetts [48]. Meredith and Lesser [29] found 92% reduction in larval densities and 78% reduction of finding mosquito larvae (i.e. frequency) on average summarizing the results of a 28-year OMWM implementation in Delaware. James-Pirri et al., [34] also observed reduction in both the proportion of time the mosquitoes were present and the larval density, although these trends were somewhat obscured by the parameter variability at the control sites. In our study, the frequency of finding larvae in the treatment areas post-project was reduced by ~70% on average, while remaining essentially identical in the control areas pre and post-project. Although the magnitude of this change was somewhat lower than that typically reported in the literature for an OMWM project, this reduction led to marked differences in spatial patterns of larval distribution on the ground. Statistically significant clusters of larvae were no longer present in the treatment areas, but consistently remained in place in the control areas post-project. Moreover, most residual breeding in the treatment areas post-project did not overlap with the pre-project larval habitat, but occupied a new niche atop or near the filled-in ditches created to restore the marsh surface. This finding highlights the potential negative consequences of marsh restoration for mosquito production if new larval habitat is generated during the process. Some of the important mosquito vectors in our area such as *Cx. salinarius*, can successfully utilize both heavily disturbed and relatively pristine marshes [7], which may result in an increased mosquito production from restored areas under favorable environmental conditions. However, even with almost complete elimination of the grid ditching system, only about 21% of the filled-in locations supported new larval habitat suggesting that the majority of the grid ditches contributed very little or none to mosquito larval habitat. For that reason, restoring the marsh surface by removing grid ditching is conceivable if proper surveillance measures to identify problem areas are implemented. GPS based monitoring and GIS/geostatistical techniques such as those described in this article represent crucial components of any surveillance program if a comprehensive project evaluation is required.

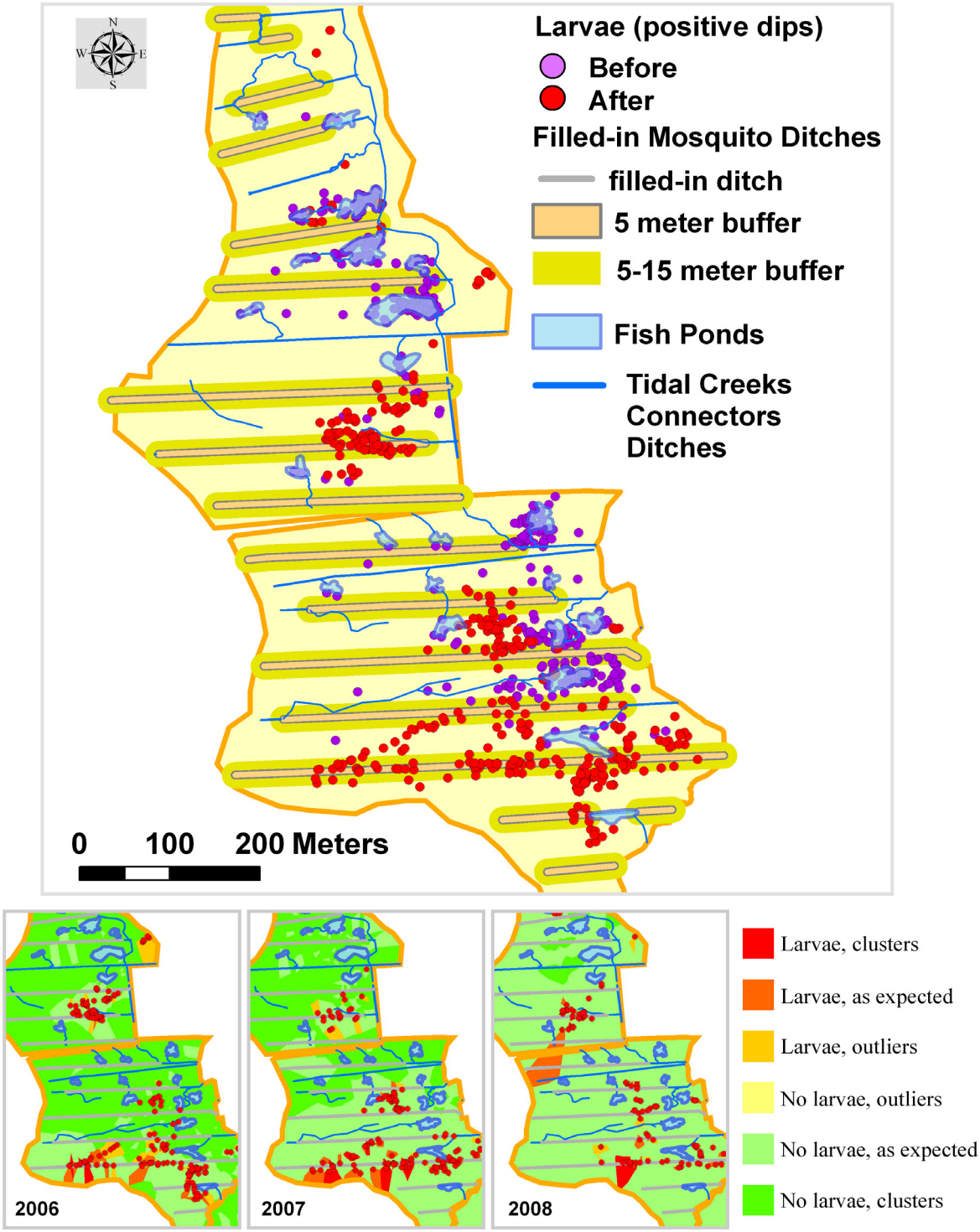

Figure 9
Post-project problem areas. Likelihood of finding larvae (from high to low) by year (2006–2008) based on Anselin Local Moran's I is indicated by the color ramp.

Table 5: Mean number of larvicide applications per month (May–September) in 2001–2008. Post-project values (treatment areas) are indicated in bold print.

Year	2001	2002	2003	2004	2005	2006	2007	2008
Area1	2.4	1.6	2.0	1.8	**0.2**	**0.4**	**0.2**	**0.6**
Area2	2.4	1.6	2.0	1.8	1.0	**0.8**	**0.8**	**0.6**
Area3	2.4	1.6	2.0	1.8	1.0	2.6	1.4	1.6
Area4	2.8	1.4	2.8	1.8	1.4	2.6	2.2	1.6

Despite the residual mosquito larval habitat in the treatment areas, the OMWM ultimate goal of significantly reducing larviciding while providing sufficient mosquito control was accomplished. The number of larvicide applications was lowered by about 74% in the treatment areas following treatment. This was due to two factors that directly affected the pesticide application criteria: the larviciding threshold and the spatial extent of the mosquito breeding areas on the marsh surface. The larviciding threshold of 0.2 larvae per dip was reached less frequently in the treatment areas by approximately a factor of 2 following treatment due to fewer positive samples containing larvae. The number of larvae per positive dip (i.e. breeding intensity), however, remained similar between treatment and control areas. This observation may be attributed to highly efficient predation of mosquito larvae by killifish in the accessible areas within Areas 1 and 2, whereas locations in the same treatment areas not easily

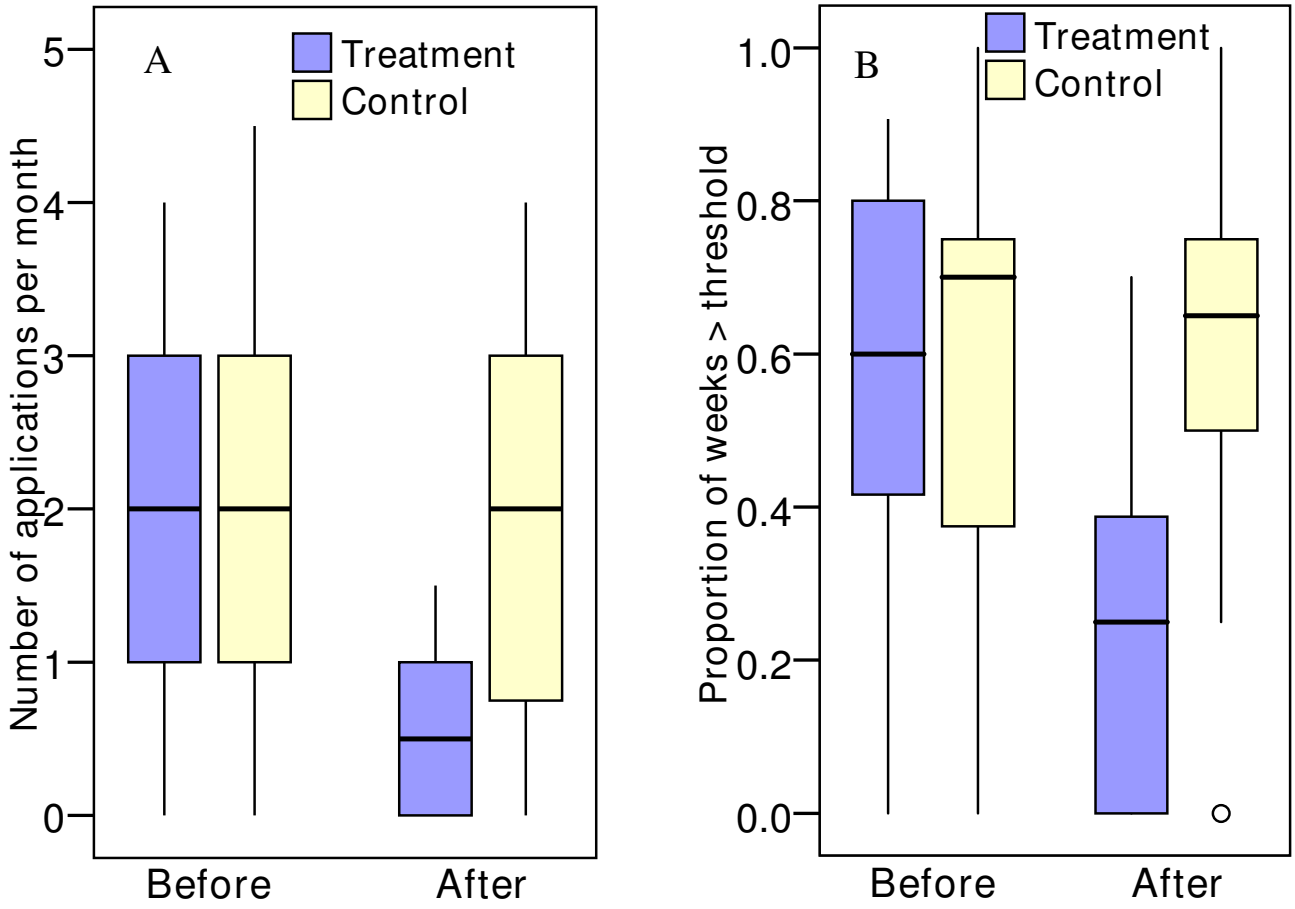

Figure 10
A) Number of monthly larvicide applications before and after treatment. B) Proportion of weeks per month when the treatment threshold (> 0.2 larvae/dip) was reached. Minimum and maximum (bar), interquantile range (box), median (horizontal line), and outliers (circle) are shown.

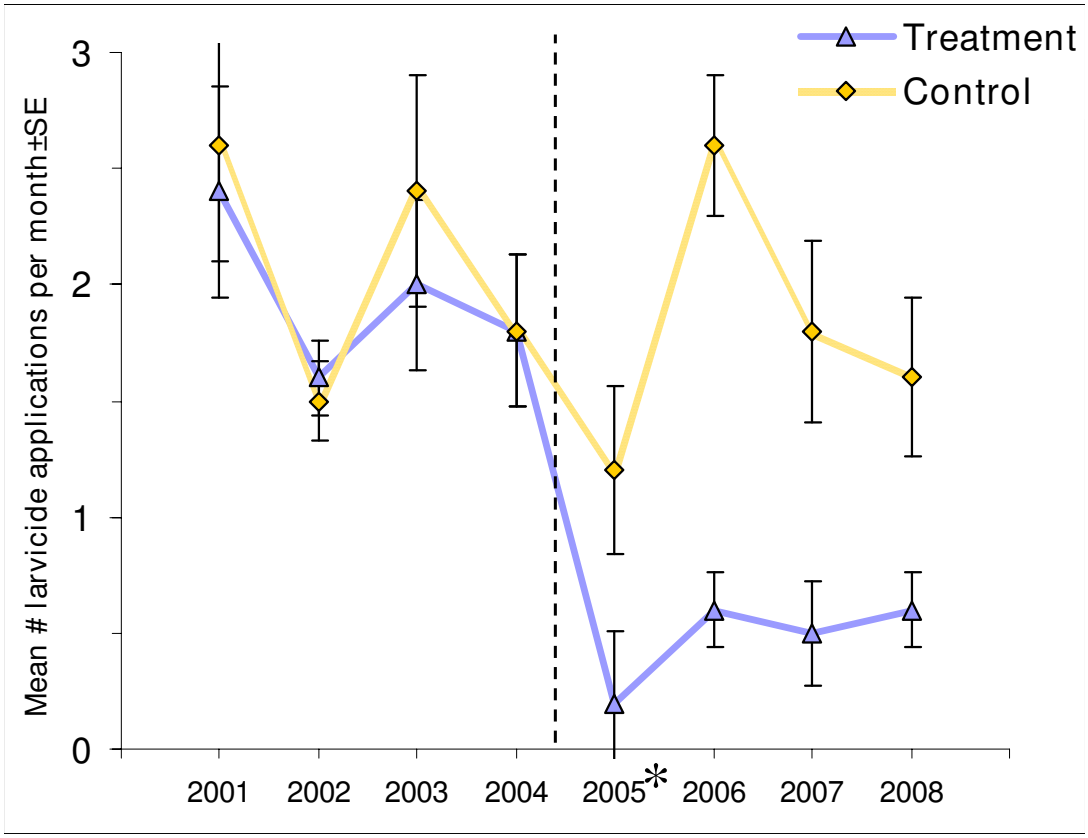

Figure 11
Mean number of larvicide applications ± SE per month (May–September) in treatment (Areas 1 and 2) and control (Areas 3 and 4) areas. Before and after is indicated by a dotted line. Dotted bars show the differences between treatment and control areas. *Area 1 was the only treatment area in 2005.

accessible by larvivorous killifish and containing larval habitat (such as the surface of the newly filled-in ditches) continued to support mosquito breeding at the similar intensity to that of pre-project. Changes in the spatial distribution of the mosquito larvae, with reduced extent and loss of clustering in treatment areas also contributed to fewer larvicide applications compared to those in the control areas (Figures 5 and 7). Although 74% reduction in number of larvicide applications is slightly lower than 90–100% reported by other investigators [29,32,49], this difference may be attributed to the expanded scope of this project (i.e. marsh restoration discussed above), lower larviciding thresholds, and more rigorous monitoring procedures.

Continuation of larviciding activities throughout the study period illustrates the difficulties in conducting large scale experiments in natural settings. As was noted previously, pesticide application may confound the results on mosquito production making their interpretation more difficult [34]. To avoid potential bias, Wertheim IMM adopted a set of criteria for larviciding triggers, which were uniformly applied to both treatment and control areas. Using these criteria, the treatment areas consistently received significantly fewer larvicide applications during the post-treatment period (Table 5). In this case, the confounding effect of larviciding would be expected to lessen the differences in mosquito production between treatment and control areas thus leading to a decrease in the before-after effect. However, the differences attributable to OMWM were not only detectable, but statistically significant. Control areas supported higher mosquito production despite retaining intact grid ditching and being subjected to 3–4 times more larvicide applications than did the treatment areas post-project. Thus, the OMWM component in this IMM project demonstrated its potential to largely replace chemical control and marsh-wide parallel grid ditching for effective larval mosquito control.

Conclusion

This study investigated the effectiveness of Open Marsh Water Management (OMWM) for mosquito vector control when combined with salt marsh restoration and invasive plant species control. Significant reduction was

achieved in the frequency of finding larvae on the marsh surface leading to loss of spatial larval clusters or "hotspots" in the areas under OMWM. In turn, these changes resulted in a significant decline of the number of larvicide applications in those areas. Random transect sampling was inadequate to assess the mosquito larval population due to a large proportion of dry "no data" sampling points. More informative targeted sampling necessitated extensive application of GPS and GIS tools to collect and analyze the data using geostatistical methods as an alternative or a supplement to the conventional inference statistics. Geographic analysis was also instrumental in identifying the residual post-OMWM larval habitat, which was largely confined to some of the restored marsh surface, albeit only in a relatively small fraction of the total. Overall, geospatial analysis proved to be a highly useful tool in evaluating the project and more completely understanding how the marsh alterations impacted mosquito larval habitats.

Although mosquito breeding was greatly reduced but not eliminated in OMWM marshes, this technique demonstrated considerable potential for effective mosquito control, which is also compatible with other natural resource management goals such as restoration, wildlife habitat enhancement, and invasive species abatement. No further interventions have been carried out in the treatment areas up to date. In the future, limited scope refinements to reduce or eliminated the remaining larval mosquito habitat may be considered based on geospatial surveillance results.

Abbreviations

BACI: Before After Control Impact; GIS: Geographic Information Systems; GPS: Geographic Positioning System; IMM: Integrated Marsh Management; OMWM: Open Marsh Water Management; USFWS: United States Fish and Wildlife Service

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IR carried out entomological field work, generated maps, performed the statistical analysis, and drafted the manuscript. TI made significant contributions to the study's conception, design, and implementation, coordinated GIS data processing and map production, and created custom software and geodatabases for the study. MED coordinated and carried out the field data collection. DVN is the principal investigator who conceived of and designed the study, directed the study implementation, and supervised data collection. All authors participated in the result interpretation and manuscript revision.

Note

The full report on Wertheim National Wildlife Refuge Integrated Marsh Management project is available at:

http://www.suffolkcountyny.gov/health/suffolkvectorplan/wdr2007.html

This website contains links to the comprehensive 2003–2007 report and the data sets of various parameters monitored during the project including mosquito larval surveillance.

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Pesticides and Public Health: Integrated Methods of Mosquito Management

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Pesticides have a role in public health as part of sustainable integrated mosquito management. Other components of such management include surveillance, source reduction or prevention, biological control, repellents, traps, and pesticide-resistance management. We assess the future use of mosquito control pesticides in view of niche markets, incentives for new product development, Environmental Protection Agency registration, the Food Quality Protection Act, and improved pest management strategies for mosquito control.

Vector-borne diseases (including a number that are mosquito-borne) are a major public health problem internationally. In the United States, dengue and malaria are frequently brought back from tropical and subtropical countries by travelers or migrant laborers, and autochthonous transmission of malaria and dengue occasionally occurs. In 1998, 90 confirmed cases of dengue and 1,611 cases of malaria were reported in the USA (1) and dengue transmission has occurred in Texas (2). Other vector-borne diseases continue to pose a public health threat. Even though the reported incidence of most of these diseases is low (in 1997, 10 cases of eastern equine encephalitis, 115 of LaCrosse, and 14 of St. Louis encephalitis [SLE]), occasional epidemics, e.g., of SLE (1,967 cases in 1975 and 247 cases in 1990, mostly in Florida [3]) have resulted in aerial applications of insecticides, primarily malathion. In addition, new vector-borne threats continue to emerge. In 1999, West Nile virus, an Old World flavivirus related to Saint Louis encephalitis virus, was first recorded in New York (4). The virus, which is transmitted by anthropophilic mosquitoes, caused a serious outbreak (62 cases, 7 deaths) and signaled the potential for similar outbreaks in the Western Hemisphere. Pesticides, which traditionally have been used in response to epidemics, have a role in public health as part of sustainable integrated mosquito management for the prevention of vector-borne diseases. We assess the future use of pesticides in view of existing niche markets, incentives for new product development, Environment Protection Agency (EPA) registration, the Food Quality Protection Act (FQPA), and improved pest management strategies for mosquito control.

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Sustainable Integrated Mosquito Management and Public Health

Mosquito control in the United States has evolved from reliance on insecticide application for control of adult mosquitoes (adulticide) to integrated pest management programs that include surveillance, source reduction, larvicide, and biological control, as well as public relations and education. The major principles of integrated mosquito management are available at a new Public Health Pest Control Manual internet website (5). Adulticides still play a vital role when flooding causes extreme numbers of nuisance mosquitoes or when outbreaks of diseases such as SLE occur.

Surveillance programs track diseases harbored by wild birds and sentinel chicken flocks; vector-borne pathogens in mosquitoes; adult and larval mosquitoes and larval habitats (by aerial photographs, topographic maps); mosquito traps; biting counts; and follow-up on complaints and reports by the public. When established mosquito larval and adult threshold populations are

exceeded, control activities are initiated. Seasonal records are kept in concurrence with weather data to predict seasonal mosquito larval occurrence and adult flights.

Source reduction consists of elimination of larval habitats or rendering of such habitats unsuitable for larval development. Public education is an important component of source reduction. Many county or state mosquito control agencies have public school education programs that teach children what they and their families can do to prevent mosquito proliferation. Other forms of source reduction include open marsh water management, in which mosquito-producing areas on the marsh are connected by shallow ditches to deep water habitats to allow drainage or fish access; and rotational impoundment management, in which the marsh is minimally flooded during summer but is flap-gated to reintegrate impoundments to the estuary for the rest of the year.

Biological control includes use of many predators (dragonfly nymphs and other indigenous aquatic invertebrate predators such as *Toxorhynchites* spp. predacious mosquitoes) that eat larvae and pupae; however, the most commonly used biological control adjuncts are mosquito fish, *Gambusia affinis* and *G. holbrooki.* Naturally occurring *Fundulus* spp. and possibly *Rivulus* spp., killifish, also play an important role in mosquito control in open marsh water management and rotational impoundment management. Like many fish, mosquito fish are indiscriminate feeders that may eat tadpoles, zooplankton, aquatic insects, and other fish eggs and fry (6). However, since they are easily reared, they have become the most common supplemental biological control agent used in mosquito control. The entomopathogenic fungus, *Laginidium giganteum,* has been registered for mosquito control by EPA under the trade name Liginex, but products have not become readily available. The pathogenic protozoon, *Nosema algerae,* has also not become available for technical reasons. Entomoparasitic nematodes such as *Romanomermis culicivorax* and *R. iyengari* are effective and do not require EPA registration but are not easily produced and have storage viability limitations. A predacious copepod, *Mesocyclops longisetus,* preys on mosquito larvae and is a candidate for local rearing with *Paramecium* spp. for food.

Mosquito traps (such as the New Jersey and the Centers for Disease Control and Prevention designs) have been used for monitoring mosquito populations for years. New designs using mechanical control to capture adult mosquitoes have now become available. These designs use compressed carbon dioxide, burning propane, and octenol to attract mosquitoes and fans to control air flow. The new technology is expensive: these traps may cost well over $1,000 each. Electric high-voltage insect traps ("bug zappers") with "black" or ultraviolet light sources do not provide satisfactory adult mosquito control and kill insects indiscriminately.

Pesticides

Pesticides used by state or local agencies to control nuisance or public health pests have warning labels and directions to minimize risks to human health and the environment. These pesticides are applied by public health employees who are specifically trained to follow proper safety precautions and directions for use. State or local mosquito control programs are funded by taxes and subject to public scrutiny. The environmental hazards precautionary statements on many mosquito insecticide labels state that insecticides are toxic to birds, fish, wildlife, aquatic invertebrates, and honeybees. Because of the low rates of application used to control mosquitoes and the special public health pest control training of most applicators, hazard to nontargeted organisms is limited. However, honeybees may be killed if exposed when foraging, so proper precautions are warranted. Human exposure in residential areas is also uncommon because of the very low application rates, ultra low-volume methods (ULV), treatment at night when people are indoors, pesticide applicator training, and public prenotification before application. Pesticide applicators who mix, load, and apply the concentrated insecticides use personal protective equipment to avoid exposure and closed systems to pump insecticides from storage to spray equipment.

The Federal Food Drug and Cosmetic Act (FFDCA) 21 USC 9§406 is the regulation that limits the quantity of any poisonous or deleterious substance added to food. A pesticide residue is the pesticide or its metabolites in or on raw agricultural commodities or processed food and feed. A tolerance is the maximum limit of a pesticide residue considered safe. Tolerances are relevant to adult mosquito control because wind drift may carry the pesticide over agricultural

crops where residues subject to legal tolerance requirements may occur. Crop tolerances are listed in the Code of Federal Regulations (7).

Larvicides

Detection of large numbers of immature mosquitoes in areas where source reduction or biological control is not feasible may require larvicide treatment to prevent the emergence of adult mosquitoes. Use of larvicides is less controversial than use of adulticides, although use of larvicides may lead to public concern about their effects on untargeted beneficial aquatic arthropods and vertebrates (Table).

Adulticides

Effective sustainable integrated mosquito management programs strive to prevent large

Table. Pesticides used for mosquito control in the United States

Name	Trade name	Formulation	Application	Advantage	Limitation
Temephos	Abate	G, EC	Larvae	Usually lowest cost	Nontarget effects, some resistance
Methoprene	Altosid	G, B, P, LC	Larvae	Residual briquets, nontarget safety	Cannot be certain of performance until too late to retreat
Oils	BVA, Golden Bear	Oil	Larvae, pupae	Acts on pupae	Oil film, subsurface larvae
Monomolecular film	Agnique	Liquid	Larvae, pupae	Acts on pupae	Subsurface larvae
Bacillus thuringiensis israelensis (Bti)	Aquabac, Bactimos, LarvX, Teknar, Dunks	WDG, AS, P,G,B	Larvae	Nontarget safety, Briquets control 30+ days	Short window of treatment opportunity. pupae
Bacillus sphaericus (Bs)	VectoLex	G, WDG	Larvae	Nontarget safety	Pupae, only works in fresh water
Malathion	Fyfanon, Atrapa, Prentox	ULV, thermal fog	Adults	Tolerances	OP, some resistance
Naled	Dibrom, Trumpet	ULV, EC, thermal fog	Adults	Tolerances	OP, corrosive
Fenthion	Batex	ULV	Adults	None specified	OP, Florida only, RUP, tolerances
Permethrin	Permanone, AquaResilin, Biomist, Mosquito-Beater	ULV, thermal fog, clothing treatment	Adults, clothing treatment for ticks and mosquitoes	Low vertebrate toxicity	None specified
Resmethrin	Scourge	ULV, thermal fog	Adults	Low vertebrate toxicity	RUP, no tolerance for residue on crops
Sumithrin	Anvil	ULV, thermal fog	Adults	Low vertebrate toxicity	No tolerance
Pyrethrins	Pyrenone, Pyronyl	ULV, EC	Adults, larvae	Natural pyrethrum, tolerances	May be costly

AS = Aqueous Suspension; B = Briquets; EC = Emulsifiable Concentrate; G = Granules; LC = Liquid Concentrate; P = Pellets; ULV = Ultra Low Volume; WDG = Water-Dispersible Granule; OP = Organophospate insecticide; RUP = Restricted Use Product

flights or swarms of mosquitoes through all the measures described above, but heavy precipitation, flooding, high tides, environmental constraints, inaccessible larval habitats, missed breeding sites, human disease outbreaks, as well as budget shortfalls, absent employees, or equipment failures, may necessitate use of adulticides (Table). Some local mosquito control programs would use an integrated program if they had adequate resources, but may be so limited in funding and personnel that adulticiding trucks are the only means of mosquito intervention.

Effective adult mosquito control with insecticides requires small droplets that drift through areas where mosquitoes are flying. The droplets that impinge on mosquitoes provide the contact activity necessary to kill them. Large droplets that settle on the ground or vegetation without contacting mosquitoes waste material and may cause undesirable effects on nontargeted organisms. To achieve small droplets, special aerial and ground application ULV equipment is used. Insecticides are applied in a concentrated form or technical grade and at very low volumes such as 1 oz (29.6 mL) per acre. Typically, aerial applications produce spray droplets of 30 to 50 microns measured as mass median diameter, with $\leq$2.5% of the droplets exceeding 100 microns. Ground ULV applicators produce droplets of 8 to 30 microns, with none >50 microns mass median diameter. Large droplets of malathion, naled, and fenthion in excess of 50 to 100 microns can damage automotive or similar paint finishes.

Adulticide applications, particularly aerial applications and thermal fogging, are quite visible and contribute to public apprehension. Ground ULV application may be less alarming than aerial application but is not effective over large or inaccessible areas. Preferable air currents for ground applications are 3.2 kph to 12.9 kph and not in excess of 16.1 kph. Excessive wind and updrafts reduce control, but light wind is necessary for drifting spray droplets. With insecticide application by air using high-pressure pumps of 2,500 lbs psi, special nozzles, proper aerial application altitude and wind drift, mosquito control is achievable for several miles downwind with minimal spray deposit below the aircraft, as a result of improved atomization of the insecticide. This technology is being developed and needs validation under different conditions with different mosquito species before it can be universally used. Thermal fogging, which was commonly used before ULV applications became prevalent, continues to be used in a few areas in the United States and is still widely used in other countries. The insecticide is diluted with petroleum oil and vaporized with heat into a dense, highly visible fog of very small uniform droplets, which allows tracking the plume downwind to target areas. Although this fog reduces visibility, it may also penetrate vegetation better than a ULV application. Small electric or propane thermal foggers are available for consumer use in retail stores at a cost of approximately $60.00.

Adult mosquitoes are easily controlled with insecticides applied at extremely low rates. For example, malathion is applied at 3 fl oz per acre (219.8 mL/ha) for mosquitoes, while the rate for agriculture is as much as 16 fl oz per acre (1,172 mL/ha).

Insecticide Resistance

Vector resistance to certain larvicides and adulticides has occurred periodically. Failure of mosquito control indicating resistance must be verified by laboratory analysis or use of test kits because other factors (improper equipment calibration, dilution, timing and other application errors, off-specification products, climatic factors) can prevent insecticides from providing satisfactory control in the field. Resistance may occur between insecticides within a class or could be passed from immature to adult stages subject to the same insecticidal mode of action. Additionally, different species of mosquitoes may inherently vary in susceptibility to different larvicides and adulticides. Insecticides with different modes of action can be alternated to prevent resistance. Even though source reduction and use of predators such as larvivorous fish are also used for sustainable integrated mosquito management, only two chemical classes of adulticides (organophosphates and pyrethroids) with different modes of action are available. Biological controls (including birds and bats) may be present, but often not in sufficient numbers to provide satisfactory alternative control, particularly in coastal areas where salt-marsh mosquitoes are abundant or when human disease outbreaks occur. Therefore, sustained integrated mosquito management requires alternative use of different classes of insecticides, in conjunction

with resistance monitoring, source reduction, biological control, and public education.

Repellents

Insect repellents, primarily N,N-diethylmetatoluamide (DEET), are used to prevent nuisance bites from mosquitoes (as well as ticks, biting flies, and mites) and may aid in lowering disease transmission from these pests. However, they should not be relied upon to prevent disease transmission, particularly where Lyme disease or encephalitis are endemic or malaria, yellow fever, or other vector-borne diseases are prevalent. Repellents, mosquito coils, and permethrin clothing treatment products are subject to EPA pesticide registration performance requirements (8). Information on safe use of repellents is located at the EPA Office of Pesticide Programs website (9). Citronella and its oil for mosquitoes and 30 other active ingredients are exempted from EPA pesticide registration (10). However, some of these products may not be efficacious.

Future of Public Health Pesticides

The past decade has seen a sharp rise in public apprehension concerning the use of pesticides, although state and federal regulations are well established for the assessment and mitigation of their human and environmental risks. Response to public concern over safety of pesticides prompted the FQPA, which includes provisions to protect availability of public health pesticides. However, public health pesticides are in jeopardy for the following reasons: In the United States, mosquito control programs are often for nuisance rather than disease vector control and not many insecticides are registered for this use. None of the mosquito adulticides commonly used were developed recently; their registrations are up to 44 years old. Mosquito control is only a niche market compared with agricultural pest control, which includes pesticides for use on corn, soybeans, and cotton, as well as the high-profit home, garden, and structural pest control markets. As pesticide companies have merged to form multinational conglomerates, the most profitable markets are those that drive corporate decisions. At present, it may require $50 million or more to develop and register a new pesticide with EPA. Furthermore, several years of the patent life elapse before costs are recouped and profits accrue.

Vector control uses of existing pesticides, particularly adulticides, often follow agricultural registration and commercialization as a means of expanding sales into new markets. Performance data are not usually required for registration of agricultural pesticides, but these data are required for registration of public health pesticides. For mosquito control, these data are often obtained under an experimental use permit, which requires application to EPA, submission or reference to a portion of the pesticide registration requirements according to CFR 40 § 158 Data Requirements for Registration and Reporting (7,8). Testing for mosquito adulticides or larvicides is typically done by universities and mosquito control or abatement districts, although it may be done by companies or state or federal research organizations, such as the Department of Defense or the U.S. Department of Agriculture. In addition to defining dose rates, formulations, environmental variables, and effects that must be accommodated, testing under an experimental use permit provides a means of market introduction through user and customer experience, presentations at professional society meetings, and journal publications.

Pesticide marketing often involves distributors or dealers who specialize in the market if the manufacturers do not deal directly. Profit margins that add to price are required by distribution chains. Public agencies solicit competitive bids for pesticides, which squeeze margins further, thus affecting marketing incentives. Mosquito adulticides are used at very low rates of active ingredient per acre, which limits sales volumes and margins. Some seasons have few mosquitoes, so sales are low. Product liability also plays an important role in reducing incentives because of possible personal and class-action lawsuits or court injunctions against pesticides applied over populated areas.

The Federal Insecticide, Fungicide, and Rodenticide Act and FQPA

The Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136 and FFDCA were amended by the FQPA of 1996. Amendments pertinent to mosquito nuisance and vector control include the following: review of a pesticide's registration every 15 years; expediting minor use registrations; special provisions for public health pesticides; aggregate (all modes of

exposure from a single pesticide) and cumulative (all pesticides with the same mode of action) risk assessments; an additional safety factor of up to 10 X for children; collection of pesticide use information; and integrated pest management. Special provisions for public health pesticides include the following: risks and benefits considered separately from those of other pesticides; exemption from fees under certain circumstances; development and implementation of programs to control public health pests; Department of Health and Human Services (DHHS)-supported studies required for reregistration when needed; and appropriations of $12 million for the first year after enactment and similar funding as needed in succeeding years to carry out public health pesticide provisions of the Act. The Act describes a consultation process between EPA and DHHS before any public health pesticide registration is suspended or canceled and allows additional time for submission of data. The first group of pesticides under review are the organophosphate cholinesterase inhibitors, including temephos, fenthion, naled, chlorpyrifos, and malathion. Should risk assessments result in detection of risk of concern to the Agency, cancellation or mitigations of use may follow, as exemplified by recent chlorpyrifos and diazinon use cancellations. Risk assessments may be based on data from acute and chronic toxicology and exposure studies, models that simulate exposure scenarios, reports of adverse incidents to humans and wildlife, extrapolation, maximum label use rate assumption, and worst-case exposure scenarios.

Even though the FQPA provisions were intended by Congress to ensure that existing public health pesticide uses are not lost without economically effective alternatives, the provisions may not be adequate. If FQPA results in cancellation of major agricultural uses of a pesticide that is also used in public health, it may become no longer profitable for the manufacturer to produce small quantities for mosquito control, thus ending production of the pesticide. Since adulticides used for mosquito control were registered decades ago, the data supporting their registrations may be insufficient to meet current requirements. The substantial cost involved in updating the data required for reregistration will have to be paid by pesticide registrants or the Federal government though the authorized and appropriated funding in FQPA. Data to support reregistration done at public expense are not proprietary. Registrants need proprietary data to protect their market shares from generic pesticide competition from overseas manufacturers that can use public data to support their own registrations; therefore, they may not consider requesting public funds to pay for new data to support existing registrations. However, if generic safety studies applicable to several public health pesticides are required by EPA for all reregistrations, the data could be generated by a task force of registrants and county, state, and Federal public health agencies, which would then request public funding under the provisions of the Act.

Although the development of new mosquito insecticides, particularly adulticides, is not expected to accelerate in the near future, integrated pest management tools and techniques should improve as a result of FQPA funding and the need to control continued vector-borne disease outbreaks. Integrated pest management tools have strengths and weaknesses, and continued availability of adulticides is critical. Therefore, implementation of the public health pesticide provisions of FQPA must include substantial comparative risk-benefit analyses of the significance of vector-borne disease impacts versus potential human and environmental toxic effects of pesticides used to control public health pests, both in the USA and other countries affected by EPA pesticide regulatory decisions. Public information and legislative campaigns have also become necessary to preserve the availability and use of pesticides for disease vector control as FQPA has been implemented and with the concurrent spread of West Nile virus.

Dr. Rose is an arthropod biotechnologist with the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture.

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Capture success of little brown bats (*Myotis lucifugus*) feeding on mosquitoes

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Abstract

A small colony of little brown bats *Myotis lucifugus* was observed feeding on mosquitoes (Culicidae) inside a barn near Fairbanks, Alaska in June 2000. Observations were made visually and with a bat detector. All capture attempts were associated with feeding buzzes, indicating that the bats used echolocation to detect and track the insects, although the light was bright and the insects were easily seen by us. The capture efficiency of the bats was 92% ($n = 100$). The insects did not show any defensive behaviour in response to approaching bats.

Key words: Alaska, echolocation, mosquitoes, *Myotis lucifugus*, predation

INTRODUCTION

Using a bat detector and a stop watch, the efficiency by which echolocating bats catch insects under natural feeding conditions can be estimated by counting so called 'feeding buzzes', i.e. sharp increases in the pulse repetition rate that is associated with the tracking and capture of an insect (Griffin, Webster & Michael, 1960). If the exploited insects can be identified and weighed, the food intake rate of the bats may be estimated. However, this technique requires either that each capture attempt can be assumed to result in a successful catch or that the error can be estimated by some other means. Unfortunately, distinguishing sucessful from unsuccessful capture attempts by acoustic means is not easy, mainly because there usually does not seem to be any reliable characteristic of the pulse sequence resulting in a successful catch (Houston & Jones, 2002).

Occassionally the outcome of interactions between bats and large insects have been estimated by direct observation in good light conditions. Generally, the capture success of aerial-hawking bats (*Lasiurus* spp., *Pipistrellus* spp. and *Eptesicus nilssonii*) hunting tympanate moths around street lights is about 30–40% (Dunning *et al*., 1992; Rydell, 1992; Kalko, 1995) and a similar efficiency (36%) applies to *E. nilssonii* feeding on lekking ghost moths *Hepialus humuli* (Hepialidae). Ghost moths are deaf to bat calls, but the displaying males compensate for this by employing 'acoustic concealment', i.e. staying close to clutter-producing vegetation (Rydell, 1998).

We here report on the efficiency by which little brown bats *Myotis lucifugus* capture mosquitoes (Culicidae) in open air. In this situation the capture success of the bats may be expected to be relatively high, because: (1) mosquitoes fly slowly and, as far as is known, they are deaf to bat calls; (2) the detection and tracking task may be relatively easy when there is little or no interfering clutter.

STUDY SITE AND METHODS

The observations were made inside a 'Quonset Hut' at Salcha near Fairbanks, Alaska. The hut, measuring *c*. 7×27 m and *c*. 4 m high, was previously used for storing potatoes and until recently as a garage. It was heated by a stove, near which the maternity colony of little brown bats, previously consisting of *c*. 200 individuals, resided. The bat colony remained in the same spot in June 2000, but apparently consisted of only a few individuals. The decline of the colony coincides with the disuse of the hut as a garage, and, most importantly, with the fact that it is no longer heated. Located at 65 °N, this bat colony is one of the most northern in the New World (Parker, Lawhead & Cook, 1997). Its feeding biology has been studied in some detail previously, and the bats were reported to feed predominantly on dipterans, including mosquitoes and moths (Whitaker & Lawhead, 1992).

As we entered the hut in the evening (10 June 2000) to observe and possibly count the bats, at least 3 bats

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emerged from inside the roof and started to hunt the mosquitoes that we had not deliberately attracted into the hut. By moving towards the open entrance of the hut, we ensured that mosquitoes were continuously attracted from the outside by our presence. At the entrance, the mosquitoes and the bats were well illuminated by the light from outside. They were thus easy to observe against the dark inner part of the hut. Remaining in this spot at the entrance for 2 h allowed us to continuously observe interactions between bats and mosquitoes at a distance of 1–3 m (between 23:30 and 01:30 Alaska summer time). Our observations were aided by a heterodyne bat detector (Pettersson D-200) tuned to 35–40 kHz.

RESULTS AND DISCUSSION

No bats were observed leaving the hut; all feeding that was observed took place inside. The bats were possibly hindered by the bright light conditions prevailing outside in clear weather. The bats typically flew back and forth inside the barn, often on figure-of-eights, attacking one insect at each turn near the entrance. There were between one and three bats flying inside the hut at the time, but usually only one, or sometimes two that were hunting actively. When more than one bat was active, much of the time was spent with one bat chasing the other or the others. This was most likely a territorial behaviour, perhaps in response to competition for the limited hunting space or to acoustic interference from the other bats (Racey & Swift, 1985; Rydell, 1986).

The outcome of 100 attacks against mosquitoes was recorded, none of which was identified to species. In 92 (92%) attacks the insect was caught by the bat, and in eight attacks it was missed. In addition, we recorded 10 attacks, all successful, against individuals of a tiny moth *Phyllocnistis populiella* (family Gracillaridae) (wingspan *c*. 5 mm), an outbreak species that occurred in abundance in the vicinity of the hut and which occasionally turned up at the entrance. No bats were observed feeding on other prey.

The capture attempts were always associated with feeding buzzes, indicating that the bats consistently used echolocation when tracking and catching the insects. Both the mosquitoes and the moths typically flew slowly and without any rapid changes in the flight course, and no evasive responses from any of the insects were observed when bats approached them. This was as expected, because neither culicid dipterans nor gracillarid lepidopterans are known to possess ultrasonic hearing organs or any other morphological defence against bats.

Myotis lucifugus, and other species in the genus, typically feed on small flies (Belwood & Fenton, 1976; Fenton & Morris, 1976; Vaughan, 1997). Although mosquitoes are not the dipterans most frequently eaten by these bats, they are taken when presented to *M. lucifugus* in captivity (Griffin *et al*., 1960). They are also known to be exploited by wild colonies of *M. lucifugus* in Alaska (Whitaker & Lawhead, 1992) and elsewhere (e.g. New Hampshire: Anthony & Kunz, 1977). Small flies seem to be caught much faster and with higher success rates than for example larger moths by aerial-hawking bats, as tentatively suggested by Kalko (1995), and this observation may help explain the apparent 'preference' for small flies even in the presence of larger insects (e.g. Swift, Racey & Avery, 1985; Barclay, 1991).

The bats observed seemed to prefer foraging inside the hut even though insects were probably more abundant outside. During this time of the year, when darkness does not occur at 65 °N, we have observed *M. lucifugus* forage in the shadow of willows (*Salix* spp.), overhanging slow-flowing river channels and ponds (Parker *et al*., 1997). A similar behaviour is sometimes shown by northern bats (*Eptesicus nilssonii*) in northernmost Scandinavia (69 °N), where they either forage in the shade beneath the tree canopies or, alternatively, feed at high elevation (typically 50 m or more). Particularly in clear weather, *E. nilssonii* consistently avoid feeding on mountain slopes that are lit by the midnight sun, and they ususally fly straight towards the shaded side of the valley. They also concentrate their feeding activity to a short period (*c*. 2 h) around midnight (Rydell & Strann, 1992; Speakman *et al*., 2000; J. Eklöf pers. obs. during July 2000). Hence, like *E. nilssonii* in northern Scandinavia, foraging *M. lucifugus* in Alaska typically avoid open areas near the ground, and we believe that this is a response to an increased risk of being caught by predators, particularly hawks and falcons, under bright light conditions.

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RESISTANCE IN CONSTANT EXPOSURE LIVESTOCK INSECT CONTROL SYSTEMS: A PARTIAL REVIEW WITH SOME ORIGINAL FINDINGS ON CYROMAZINE RESISTANCE IN HOUSE FLIES

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ABSTRACT

Insecticide resistance development in three continuous exposure livestock insect control systems is discussed. These include residual pyrethroid sprays and cyromazine feed-through for house flies, and insecticide cattle ear tags for horn flies. All three of these systems selected for resistance problems in the field within two years. Other discontinuous use patterns of these same chemicals have been used on the same insects in the field and selected for much lower or no resistance. The conclusion is that these systems waste the resource of insect susceptibility, especially with mobile insects with short generation time such as the flies discussed here.

RESUMEN

Se discute el desarrollo de resistencia a insecticidas en tres exposiciones contínuas de sistemas de control en ganado. Estos incluyen rocios residuales de piretroides y de criomisina "alimentos-a-través" para la mosca común, e insecticidas en etiquetas puestas en las orejas del ganado para los tábanos. Estos tres sistemas seleccionaron problemas de resistencia en el campo en dos años. Otros patrones de uso descontinuados de estos

mismos productos químicos se han usado contra los mismos insectos en el campo y seleccionados con poca o sin resistencia. Se discute y se concluye que estos sistemas malgastan las fuentes de susceptibilidad del insecto, especialmente cuando los insectos son movibles y de generaciones cortas tales como tienen las moscas.

In the last several years constant exposure and slow release insecticide formulations have been widely used in controlling pests associated with animal production in the United States. These include residual permethrin sprays and automatic spray systems for house fly adults (*Musca domestica* L.), insecticide impregnated cattle ear tags for horn flies [*Haematobia irritans* (L.)], and cyromazine feed-through as a house fly larvicide.

These low maintenance treatments have been extremely popular since they require very little labor and management. Producers can initiate and forget them. These treatments have all been highly effective on the susceptible insect populations initially encountered. But insecticide susceptibility is lost rapidly with these methods.

These treatments are persistent or, because of constant release (slow release), give constant control as a persistent insecticide would. This is (of course) by design, but persistent insecticides have been shown to select for resistance more rapidly in the field (Brown 1971, Georghiou & Taylor 1977). Taylor et al. (1983) stated that resistance develops faster with a long-lived residue than with a rapidly decaying one. Also, these treatments generally deposit a relatively low dose or degrade slowly so that a marginally effective residue is present for longer periods of time than is the case with space sprays or shorter-lived treatments. The relatively low dose associated with slow release or constant exposure systems may kill fully susceptible individuals but allow survival of heterozygous resistant individuals, causing resistance to be functionally dominant and selection to proceed rapidly (Georghiou 1980). This has been well documented with pyrethroid cattle ear tags (Roush et al. 1986, McDonald et al. 1987) where all susceptibles are killed by tag residues on cattle, but many heterozygous and all fully resistant horn flies live.

Permethrin resistance in house flies is a widespread phenomenon in North America resulting from residual premise sprays and/or automatic spray systems which operate frequently and select more or less continuously. These systems initially gave excellent control. Two to four residual permethrin sprays gave full season house fly control. Lancaster & Simco (1975) reported that a residual permethrin treatment applied (accidentally) with a 1.4 mg AI per 28 m^3 aerosol gave 100% kill of house flies at 27 wk. Combs (1978) found that an 800 mg/m^2 residue of permethrin on galvanized metal gave 100% house fly control for 123 days or more. This residue on unpainted wood gave similar control for 49 days or longer.

But resistance to these types of treatments was reported within one year after use began in Canada (Harris et al. 1982) and after two years of use in Georgia (Horton et al. 1985). This type of pyrethroid use pattern was prohibited in Denmark for house fly control because the high potential for resistance selection was recognized (Skovmand & Keiding 1979). Where persistent formulations were banned in Denmark, bioresmethrin space sprays are still effective and resistance is very low where it does occur (Keiding 1986).

Although supporting data are not available, residual premise sprays of permethrin were probably better selectors of resistance than frequent (automatic) space spray of the same material. This is because the residue would decay over a long time interval. Near the end of this interval the residue would be adequate to kill fully susceptible house flies but not heterozygous resistant ones; and essentially all genes for resistance reside in the heterozygous situation in an unselected population (Georghiou 1980).

Ear tags impregnated with insecticides were highly effective in controlling horn flies on cattle when first introduced (Ahrens 1977, Ahrens & Cocke 1979, Schmidt & Kunz 1980, Sheppard 1980). Prior to the use of insecticide impregnated cattle ear tags insecticide resistance in horn flies was not perceived as a problem. Only two cases of resistance were documented before ear tags were introduced (McDuffie 1960, Burns & Wilson 1963). After ear tags came into use horn flies quickly developed resistance to the active ingredients.

Demonstrable resistance to stirofos and fenvalerate occurred in horn flies after two seasons of use of these materials in cattle ear tags in Georgia (Sheppard 1983b, 1984) and with pyrethroids in Louisiana (Quisenberry et al. 1984). Horn flies in Florida developed resistance after two or three years of use of pyrethroid tags (Schmidt et al. 1985).

Insecticide impregnated cattle ear tags release a steady but decreasing dose of chemical for many months (Miller et al. 1983). This type of steady selection has been reported to select rapidly for resistance (Brown 1971, Georghiou & Taylor 1977). Currently available pyrethroid ear tags also favor rapid resistance selection in another way. They release a dose level of toxicant that is highly selective for heterozygous or resistant individuals. Roush et al. (1986) and McDonald et al. (1987) reported that susceptible survivorship is at or near zero percent while survival of heterozygous resistant horn flies is significantly higher (3-21%) even with very fresh tags. Heterozygous survival should be greater as tags age and doses decrease. Tags used in these tests show a seven fold decrease in permethrin release over 120 days (Miller et al. 1983). McDonald found that even four fresh permethrin cattle ear tags per steer did not kill all heterozygous resistant horn flies. Thus, low tagging rates of one tag per cow or less were probably not important in rapid resistance selection as has been speculated by some entomologists. Homozygous resistant horn flies survived at essentially 100% when on steers with one or two tags (McDonald et al. 1987).

But all this only shows that pyrethroid resistance developed rapidly in treated horn fly populations and does not show conclusively that the slow release (tag) is a large part of the resistance selection problem. Rapid development of stirofos resistance in horn fly populations treated with Rabon® Cattle Ear Tags (Sheppard 1983b) does incriminate the tag. But Quisenberry et al. (1984) reported that there is strong cross-resistance between DDT and the pyrethroids which may predispose them to become pyrethroid resistant. This predisposition alone may have led rapidly to pyrethroid resistance.

To more clearly define the role of tags in this resistance selection problem Sheppard (1987) conducted a two year study. Fenvalerate sprays or one or two fenvalerate tags per cow were used for horn fly control in each of three nearby areas. Resulting resistance factors (RF) were significantly lower in flies on the spray treated cattle (maximum RF = 31) than in the areas where one or two tags per cow were used (maximum RF = 63 and 183 respectively). Total fenvalerate deposited on the cattle each season with the spray (0.6g) was intermediate to the amount deposited by one (0.4g) or two (0.8g) tags. The steady low dose from the tags clearly selected for resistance more than the very high, but intermittent, dose of the spray treatment. Initially each spray treatment killed even highly resistant horn flies. But as the spray deposit aged relatively rapidly, susceptible immigrant horn flies could survive on the cattle near the end of a spray cycle, mate, and add susceptible genes to the population. The steady release from the tag killed susceptible flies as they entered the herd, so no susceptible mating and dilution of the resistant gene pool was possible. In other tests (Sheppard 1987), horn fly populations which had been uncontrollable with tags were readily controlled with sprays delivering the higher initial dose (ca. 37 times a two-tag treatment). Also, RF's were observed to drop late in a spray cycle, due to the potential for susceptible survival which cannot occur with the slow-release tag (Sheppard 1987).

Cyromazine as a poultry feed-through for house fly larval control in the manure is another constant exposure system used to control a pest of livestock production. This material was initially marketed by Ciba-Geigy Corporation as a 0.3% Larvadex® premix for addition to caged layer feed at 0.5 kg per metric ton, producing a 1.5 ppm rate in the feed. Initially this rate was highly effective. In fact before its commercial introduction in 1982 Hall and Foehse (1980) reported that 0.8 ppm cyromazine gave 100% reduction of house fly eclosion when exposure was initiated at the egg stage. It was also shown to give 100% control at 0.75 ppm in a field trial in Georgia prior to registration. The 1.5 ppm rate was used successfully all across the U. S. from ca. June 1982 to August 1983. Larvadex registration was cancelled by the U. S. Environmental Protection Agency in August 1983 pending the resolution of some unfavorable results of mammalian toxicological studies. In January 1984 Bloomcamp et al. (1987) collected house flies from a caged layer operation where cyromazine premix had been used for two seasons. This strain was shown to be cyromazine tolerant. Subsequent selection for 15 generations produced 69.8-fold resistance. Registration and commercial use began again ca. May 1985. Larvadex was again marketed as a 0.3% premix to be mixed at 1.5 ppm in feed for 1985 and 1986. In 1985 and 1986 house fly control with Larvadex was less than desirable at some caged layer operations. In a Mississippi facility monitored by one of us (DG) control had been excellent in 1982 and 1983. Suppression of fly breeding appeared to be complete. When cyromazine use commenced again in 1985 control was judged only good. In 1986 control had deteriorated further and was judged fair. Many larvae pupated but the majority of these were deformed. However, some did emerge as adults and control was unsatisfactory.

Eight bioassays of house fly larvae from this population in 1986 provided data to calculate resistance factors (RF) of 9-21 ($\bar{x} = 14$). In another case eight bioassys of a Georgia population where cyromazine control was failing in 1986 yielded an average RF of 4.2. In 1987 a 3.3-fold increase was made in the labeled rate of cyromazine (1.0% premix) and this produced 5.0 ppm cyromazine in the feed. Coupled with this formulation change Ciba-Geigy Corporation recommended a resistance management program of intervals of nonuse of cyromazine each year and substitution of alternate control methods in these intervals when house fly numbers were excessive. This higher rate of cyromazine was effective in eliminating fly breeding at the Mississippi operation where control problems were evident in the resistant house flies in 1986.

This renewal of efficacy by a rather modest increase in rate may be associated with the relatively high slope of the dosage mortality response line for cyromazine. The average slope of these regressions ($N = 8$) for the resistant Mississippi house fly population was 3.0 and at this slope they responded over a ca. 140-fold range (0.1-99.9% mortality). In contrast, early in an episode of pyrethroid resistance in horn flies (Sheppard 1987) the average slope was 1.1 and a corresponding range was 333,000-fold. Ear tags that were several-fold more potent and increased tagging rates had little increase in effect. These laboratory data are not absolutely applicable to the field situation but these response ranges do indicate that house flies are less adaptable (phenotypically diverse in response) to cyromazine than horn flies were to pyrethroids by a large factor (333,000-fold $\div$ 140-fold $\cong$ 2,300).

A sprayable cyromazine formulation was available for early preregistration testing but had not been labelled. This material was promising in early testing in Georgia. In 1986 a resistance selection trial was initiated to compare rate of resistance selection between the 1.5 ppm feed-through and the spray formulation. The spray produces a higher concentration of cyromazine in the manure when applied at the label rate of 4 liters 0.1% spray per 10 m^2, approximated in the following trial. This was calculated to be 22.5 ppm in the sprayed treatment if one assumes mixture with 2 weeks accumulation of manure. A 2 week period was chosen for this discussion of concentration, but this

treatment is usually effective for 2 months or longer. The initial concentration of this spray treatment on the manure would be much higher than 22.5 ppm before the cyromazine became mixed and diluted by accumulations of fresh manure and possibly by larval activity. Conversely, the 1.5 ppm cyromazine in the dry feed would be somewhat less in the manure, having been diluted by water intake of the hen, even if no other losses occurred. We reasoned that the much higher spray rate would be less likely to favor heterozygous resistant individuals (Georghiou 1980). Also the spray, as opposed to the feed-through, would be inherently discontinuous and therefore should have been a poorer resistance selector. Therefore, we initiated a fioeld test to test the hypothesis that the cyromazine spray would be less selective for resistance in house flies than the feed-through.

Materials and Methods

Four caged layer operations in southeastern Georgia were used in this trial in 1986 and 1987. Cyromazine had not been used at any of these sites within the memory of the operators. Two sites received the 1.5 ppm cyromazine feed-through treatment. These operations were to have received treated feed on an "as needed" basis for fly control and a "one week on, one week off" schedule. But communication with the feed mill incorporating the cyromazine into the feed was poor and these schedules were not maintained. One operation received the 1.5 ppm cyromazine treated feed for the following intervals: 7/8-8/12, 9/3-9/25, 10/2-10/14, 10/22-10/28, 11/5-11/11, 11/18-11/26, and 12/3-12/17 in 1986. This represented 110 days when treated feed was fed and this site is referred to as the 110-day feed-through site. The other feed-through treated operation received treated feed for the intervals of 8/18-9/2, 9/9-9/16, 10/22-10/27, 11/16-11/26, and 12/9-12/12 in 1986 which amounted to 45 days. This site is referred to as the 45 day feed-through site. A third operation was sprayed with a 0.1% cyromazine solution at ca. 4 liters per 10 m^2 manure accumulation under the hens on 7/10 and 10/1 in 1986. The fourth operation received no cyromazine treatment and served as a check. An adulticide bait was provided at this operation to be used ad libitum. Black soldier flies (*Hermetia illucens*) were present at all these sites and a very dense population developed at the untreated operation. Larvadex treatments eliminated soldier flies at the other sites. House fly adults and/or larvae were periodically collected at each site and colonized in the lab. Larvae from these colonies were bioassayed for response to cyromazine.

The laboratory bioassay used sequential dilutions of cyromazine in Purina® fly larvae media. Using an upper rate of 1.0 to 5.0 ppm (depending on the expected degree of resistance) the cyromazine was applied to 60.0 g of media in 150 ml styrofoam cups. Thirty-five two-day-old larvae were added to each cup; the cups were covered with paper towelling secured by a rubber band and held at ca. 27°C. Larvae of a known susceptible laboratory strain were tested concurrently. About eight rates of cyromazine were used per test, often ranging from 1.0 to 0.059 ppm. Each rate had 2/3 the concentration of cyromazine of the former, and one cup per rate was used per test.

Flies were allowed to continue development, pupate, emerge and die confined in the cups. Counts were made of flies that successfully emerged and percent mortality was determined by rate. Mortality was regressed on rate of cyromazine by probit analysis (Daum 1970) and the LC_{50} estimated. The LC_{50} of the field population was divided by that of a cyromazine susceptible colony to derive a RF. Statistical differences in RF's were determined by non-overlap of 95% confidence intervals of the LC_{50}'s of the test populations being compared.

Adult house fly numbers were sampled continuously with 8x13cm "spot" cards (Axtell 1970) and larval numbers and activity were estimated visually and recorded periodically at each operation. Spot card counts were not available for the 45-day feed-through

site for most of the study since the integrator stated cards would make the hens nervous and reduce egg production.

Results and Discussion

Spot card count averages did not differ greatly among cyromazine treatments, but counts were lowest for the sprayed operation. Posttreatment spot card counts at the soldier fly dominated check operation were lower than any cyromazine treatment (Table 1). Posttreatment at the check was defined as the period after soldier flies became abundant since these house fly competitors were apparently the main control factor there. Larval house fly numbers were judged low or nonexistent at the check after soldier flies became established in June (Table 2). Cyromazine use was not initiated until mid-August at the 45-day feed-through site. Soldier fly larvae were abundant there until that time and controlled house fly production (Table 2). These observations are consistent with earlier findings that dense soldier fly larval populations can exclude house fly breeding (Furman et al. 1959, Tingle et al. 1975, Sheppard 1983a). Bradley & Sheppard (1984) found that wild type house flies laid few or no eggs where soldier fly larvae were (respectively) moderately abundant or abundant. House fly collections sufficient to monitor for cyromazine response levels at the check were not possible until late October due to the control exerted by the dense soldier fly population. Few adult house flies were present and no larvae could be found from August to mid-October (Table 2). We suspect the adults present there were immigrants since no larvae were found.

We experienced difficulty getting consistent results in toxicological tests with cyromazine. This is evident when one views the bottom half of Table 3 where several bioassays of four house fly populations collected on April 22, 1987 are represented. With this in mind, we have only used populations tested together on the same day for statistical comparisons to rule out day to day variation in technique. To further minimize variation in RF, we have used the annual average LC_{50} of the laboratory susceptible

TABLE 1. Spot card counts[1] in cyromazine resistance selection test, Brantley County, GA, 1986.

	1.5 ppm feed-through		0.1% Spray	Check
	110 day	45 day		
5/7-5/21	667	—	1,024	846
5/21-5/28	792	—	1,778	1,480
5/28-6/11	762	—	1,182	620
6/11-6/26	527	—	813	242
6/26-7/22	561 Treat	—	934 Treat	193
7/22-8/7	345	—	—	142
8/7-8/21	266	—	—	148
8/21-9/3	137	— Treat	208	115
9/3-9/11	500	—	225	238
9/11-9/18	631	174	540	260
9/18-10/10	633	327	551	288
10/10-10/28	540	745	318	203
Pretreat $\bar{x}$	662	—	1,146	982
Posttreat $\bar{x}$	436	415	368	203

[1]Average number fly spots per card per week.

TABLE 2. RELATIVE NUMBERS HOUSE FLY LARVAE IN CYROMAZINE RESISTANCE SELECTION TEST, BRANTLEY COUNTY, GA 1986.

	Cyromazine treatments			
	110 Day Feed-through	45 Day Feed through	0.1% Spray	Check
May 21	high	—	high	moderate
28	high	—	high	—
June 11	—	—	moderate	low[1]
26	—	abundant	—	—
July 8, 10	began treatment	—	treatment	—
22	moderate	few[1]	none	very low[1]
Aug. 7	moderately high	none[1]	none	none[1]
14, 18	low	began treatment	—	—
21	low	abundant	none	none[1]
Sept. 3	abundant[2]	low	low	none[1]
11	abundant[2]	very low	low	none[1]
18	abundant	none	abundant	none[1]
Oct. 1	—	—	treatment	—
10	abundant	moderate	very low	none[1]
15	abundant	abundant	very low cleaned out between 10/10 and 10/15	low[1]
28	abundant	—	low	low[1]
Nov. 19	low	abundant[2]	abundant[3]	low[1]
Dec. 18	moderate	moderate	moderate	out of production

[1]Noted in conjunction with dense population of black soldier fly larvae, a competitor.
[2]Pupae predominantly appear normal (not elongate).
[3]Pupae predominantly elongate.

house fly strain as the divisor, rather than the susceptible LC_{50} for that particular test. The average laboratory susceptible cyromazine LC_{50} was 0.1424 ppm in 1986 and 0.1039 ppm in 1987.

Pretreatment RF's at the four test sites ranged from 1.0 to 1.6 (Table 3). These bioassays were not conducted concurrently and so do not meet the standards we imposed for statistical comparisons. Nevertheless these RF's were all low, and the 95% confidence limits of the LC_{50}'s of each population do overlap with the other population's.

Posttreatment RF's at each site increased (Table 3). Based on bioassays of the last 1986 collection at each site, cyromazine tolerance increased ca. 180% at the 110-day site, 280% at the 45-day site, 140% at the spray site and 20% at the check. In 1986 only populations collected from the 110-day site and the spray site on September 18 were bioassayed together. Two bioassays of each of these collections were run concurrently to allow for statistical comparison. In both of these pairs of bioassays the 110-day site (feed-through) population had RF's (4.3, 6.9) significantly higher than those of the sprayed site (2.3, 1.8). Thus, the more constant, low level exposure of the feed through did select for a significantly higher level of resistance than the spray in a ten week period. When considering this rapid change recall that Larvadex feed through was registered in 1982 and use in nearby caged layer facilities undoubtedly occurred. This early use probably resulted in some preselection as evidenced by the very low RF's seen pretreatment (Table 3).

TABLE 3. CYROMAZINE RESISTANCE FACTORS IN HOUSE FLIES AT CAGED LAYER OPERATIONS CONTROLLED WITH 1.5 PPM CYROMAZINE FEED THROUGH AND 0.1% SPRAY, BRANTLEY COUNTY, GA. 1986 AND APRIL 1987.

	Feed-through		Spray	Check
1986 Collections	110 day	45 day		Jacobs
Pre-treat (Apr-June)	1.6(1)[1]	1.5(5)	1.2(2)	1.0(3)
Aug 14	3.9(11)			
Sept 3	2.4(3)			
Sept 18	4.6(6)A[2]		2.9(4)B	
Oct 10		5.8(6)		
Oct 28				1.2(1)
1986 Posttreat $\bar{x}$	3.6	5.8	2.9	1.1
4/22/87 Collection tested on:	Feed-through		Spray	Check
	110 day	45 day		Hunter
5/2/87	3.1B	3.5B	—	1.1A
5/25/87	1.9B	1.0A	0.8A	0.6A
6/18/87	—	0.7A	1.6A	—
6/19/87	—	—	1.4A	1.3A
8/12/87	2.5B	4.6A	—	—
1987 $\bar{x}$	2.5	2.4	1.3	1.0

[1]Number in parenthesis is the number of LC_{50}'s from significant regressions used to determine this avarage resistance factor for this collection.

[2]Resistance factors in the same row followed by the same letter are not significantly different ($P \leq 0.05$).

On April 22, 1987 house fly collections were made at all three cyromazine treatment sites. A new check site (Hunter) had been chosen since the original (Jacobs) check site had ceased operation and April 22 collections were made at the new site. The two feed through sites received their last treatments in mid-December. These were the last treatments necessary for fly control, as was the second spray of October 1 at the spray site. We conducted bioassays from these April 22, 1987 collections together so that comparisons would be more meaningful. Average RF's from the bioassays of all treated site collections were lower than the last collections in 1986 (Table 3), probably a response to immigration of susceptibles during the 4-6 mo. untreated period. The new untreated check was highly susceptible as had been the previous one (Table 3). Comparisons of these populations showed no statistically significant differences between the sprayed site and the check. The 110 day feed-through site was significantly more resistant than the check in both bioassay sets (5/2 and 5/25) when significant regressions allowed these comparisons. The 45-day site was significantly more resistant in one out of two possible comparisons (Table 3).

Cyromazine bioassay results were variable, but lower resistance selection by the spray treatment was clear in this trial. These results are in agreement with what we know of cyromazine resistance in the U. S. and in Denmark. In the U. S. cyromazine has been used as a feed-through and resistance began to be a problem after two years. In Denmark risk of resistance development is a consideration in pesticide registration so only the spray formulation was registered there. Cyromazine spray has been in widespread use since 1984 with no indication of resistance development (Keiding 1986 and Jorgen Jespersen, personal communication 1988).

As indicated earlier, an increased rate of cyromazine feed-through and recommendations for intermittent use seem to be regaining control where significant resistance developments in treated house fly populations had occurred. Early management of the problem and the inherently more diverse habitats of house flies over horn flies may prevent house fly cyromazine resistance from ever reaching the proportions of the horn fly pyrethroid resistance problem seen in the mid 1980's. The higher slope (shorter response range) of the cyromazine house fly system may also help make this problem more manageable. Labelling of a cyromazine sprayable formulation in the U. S. would give our producers and pest managers even greater flexibility and reduce the importance of low levels of resistance.

Three pesticide use systems which developed resistance problems after only two years of use have been outlined here. All three subjected the pest population to constant selection with a rather low dose of toxicant, or with a persistent, slowly degrading dose that was marginally effective at least in its later stages. In all three cases these two factors, 1) constant selection and 2) a low but apparently effective dose, led very rapidly to resistance problems.

Can we afford to expend the useful life of our insecticides this quickly? Given the cost of developing a marketable insecticide, the low number of these that are applicable to livestock entomology, and the essential nonretrievability of insecticide susceptibility the prudent answer seems to be "no". There certainly is a place for this type of formulation, but it seems to be an error to let this use be so general. We are trading convenience against very short use-lives. In the long-run this constant exposure approach is costly to suppliers as well as users. Whenever possible other formulations less likely to select rapidly for resistance from susceptible pest populations should be used.

Endnote

Presented at the 1987 Florida Entomological Society Meeting, Daytona Beach, FL, as part of a symposium entitled "Insect Pest Management in Livestock Systems".

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Garlic as an Insect Repellent

Louise Stjernberg; Johan Berglund

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The Past and Future of Medical Malpractice Litigation

To the Editor: Dr Mohr[1] argues that 3 medical and 3 legal factors sustain medical malpractice litigation. I believe that an additional factor is the modern attitude that values litigation above other approaches to resolving disputes. Individual and institutional members of society are increasingly litigious as is evidenced by caseload data from the National Center for State Courts.[2] Recent tobacco court cases demonstrate that the government also is suing its own citizens to resolve disputes instead of using the legislative process.

From 1984 to 1994, the number of cases filed for juvenile, domestic, criminal, and civil offenses increased by 59%, 65%, 35%, and 24% respectively.[2] The total number of cases in 1994 for these 4 categories was more than 34 million and represented 13.75 cases per year per 100 citizens, assuming a total US population of 250 million.

At the same time (1984-1994), tort filings increased 20%, from 251 983 to 303 006.[2] Besides medical malpractice, these tort cases included product liability, professional malpractice, toxic substances, premises liability, intentional injury, automobile crashes, and slander or libel.[2] Again, the diversity of these lawsuits confirms the value that individual and institutional members of the society place on litigation.

I believe that these societal values are probably more important than the values described by Mohr in sustaining litigation in medical malpractice and in many other areas as well.

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2. National Center for State Courts. *Caseload Highlights* [serial online]. Available at: http://www.ncsc.dni.us/Research/CSP/csphigh.htm. Accessibility verified.

To the Editor: Dr Mohr[1] and Mr Gostin[2] provide useful insight on medical malpractice and medical error. Other commentators also have expressed concern that malpractice litigation does not adequately reduce error in our health care delivery system.[3] However, none of these authors discuss the ethics of physicians who serve as expert witnesses.

For instance, activities such as peer review, quality assurance, and morbidity and mortality conferences are generally not discoverable in court. Thus, is it hypocritical for physicians to embrace the secrecy of quality assurance–type reviews when the error is their own or that of one of their colleagues and then also to accept payment for testifying against other physicians? I believe it is except under the following circumstances: the physician being sued has committed an act of gross incompetence or intentionally harmed the patient; the plaintiff's expert witness has never made a mistake (obviously, this exception never occurs); when the plaintiff's expert witness makes a mistake, he or she advises the patient to seek compensation (I doubt any physician would even consider this option); or the plaintiff's expert witness waives his or her right to have quality assurance–type review be nondiscoverable. (No hospital, health care organization, or malpractice insurer would allow such a waiver.) If one assumes that such behavior is hypocritical and, as such, perhaps unethical, the credibility of plaintiff's expert witnesses should be seriously questioned.

Where would this leave patients who are truly harmed by negligent care? Our current system cannot solve this or many other pieces of the medical error puzzle. Furthermore, with personal injury attorneys on one side and bean counters on the other, significant reductions in medical error will not occur without major reforms. As such, isn't it time to give enterprise liability or "no-fault" insurance a try?

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To the Editor: Dr Mohr's[1] survey of the historical bases of the origin, perpetuation, and growth of US medical malpractice litigation identifies several factors with continued relevance to the future of such proceedings.

Modern parallels of the developments underlying the emergence of medical malpractice in the 1840s suggest that complementary and alternative medicine (CAM) may soon experience its own "malpractice crisis." Medical malpractice emerged in the mid-19th century following a period of increased religiosity, greater popular attention to physical fitness and health, and food reforms—all phenomena with contemporary analogues. Similarly, practitioners from a wide variety of disciplines purveyed

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services in both periods, although the "regulation by market" of the early 1800s ("marketplace professionalism") is now largely limited to CAM. The development of medical practice standards in the 1840s and 1850s (eg, definition of a physician's duty of care to the patient)—the sine qua non for the original appearance of medical malpractice litigation—may have a contemporary analogue in recent scientific studies of complementary therapies.[2] Notwithstanding these historical similarities, malpractice litigation against CAM practitioners may not parallel the course of traditional medicine[3]: studies may discredit some disciplines, complementary therapies may not be amenable to evidence-based practice guidelines,[4] their practitioners may not have sufficiently "deep pockets" to justify lawsuits, and patients choosing CAM may be less likely to litigate because of a perceived closer relationship to their practitioners or greater personal responsibility for their choice of therapy.

Two other trends with historical roots may affect both traditional and alternative medicine. First, patients' increasing activism and the emergence of the patient-physician "partnership" or collaborative model demonstrate that patients are assuming greater responsibility for their care. This changed role[5] may justify a shift from tort-based to contract-based liability, or afford a defense for practitioners (eg, patient contributory or comparative negligence), particularly within the context of CAM.[6] Second, as an increasing proportion of the population is insured by managed care organizations, health care may be perceived as a commodity marketed by faceless profit-motivated corporations, rather than as a professional service built on a personal relationship between practitioner and patient. This changed perception may increase patients' propensity to litigate; to the extent that the perception is accurate, it also argues for enterprise liability. Significantly, both the contractual liability model and enterprise liability theory reflect the commercialization of medicine and renunciation of its professional status, the quest for which, ironically, contributed to the current state of tort-based malpractice litigation.

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In Reply: Dr Kakaiya suggests that medical malpractice litigation essentially comes with the territory in the United States, where "societal values" have encouraged recourse to the courts over other methods of dispute resolution. He further contends that those values have been growing stronger in recent decades. Surely US litigiousness is a factor in the big picture, but litigiousness in general cannot explain the current circumstances of medical malpractice. Americans were extremely litigious well before medical malpractice appeared on the scene, and the rate of medical malpractice suits rose less steeply in the years Kakaiya cites than in several earlier decades of US history.[1] Moreover, other professional groups in the United States historically have been sued far less often than physicians and do not now face major malpractice crises of their own.

For every instance of medical negligence that results in a malpractice action (as medical negligence is currently understood), at least 8 instances of medical negligence presently do not result in lawsuits.[2-4] Some estimates put that second number many times higher. From a historical point of view, the wonder is not how many litigation-prone Americans have been suing their physicians in recent decades, but how few. Current pressures on the medical malpractice system appear to have resulted less from increased rates of litigation (however measured) than from quantum jumps in settlement amounts. The latter have brought our historically ad hoc system of individual liability insurance to the edge of financial irrationality, without providing compensation to the vast majority of patients who might deserve some redress. That troubling reality underscores the need to consider overarching societal values, along with all of the other interacting dynamics in the modern malpractice tangle, in long-term historical context.

Citing what he regards as the hypocrisy of expert witnessing, Dr Migden asks, ". . . isn't it time to give enterprise liability or 'no-fault' insurance a try?" I have written elsewhere about the history of medical expert witnessing, which has long presented problems in the context of our adversarial legal system.[5] By themselves, however, those problems have never justified abandoning the system. But I do believe Migden's larger question should be taken seriously, primarily on the basis of the other dynamics I outlined in my article, particularly those involving the history of liability insurance itself.

Dr Van Oeveren reminds us that ambiguities associated with professional status remain as central to the malpractice conundrum in the United States today as they consistently have been since the 19th century.

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In Reply: Dr Migden expresses common, and understandable, frustration with the medical malpractice system in the United States. However, his implied solution, that physicians should not provide expert testimony in negligence cases, is not reasonable. In making his arguments, Migden compares expert testimony with peer review. While I certainly understand the analogy, it is not sound.

These 2 systems—medical malpractice in the courts and peer review in hospitals—are intended for different, but related, purposes. The peer review system is not designed to absolve physicians from responsibility for their negligence, although that is sometimes the result. Rather, peer review (and the associated legal privileges given to it) is intended to encourage honest and full reporting of adverse events by physicians and careful evaluation of quality by hospitals. Peer review provides an opportunity for health care organizations and physicians to learn from their mistakes and put in place systems to reduce future risks to patients.

Medical malpractice, on the other hand, is designed specifically to determine fault and allocate liability. Physicians may not like the current medical malpractice system, but the appropriate response is to seek national reform. It is surely not appropriate for physicians to engage in a concerted "code of silence," by refusing to testify in cases in which their colleagues may have acted wrongfully. It is ethical for a physician to give an honest and fully informed appraisal in a court of law by acting as an expert witness.

As for reform of the medical malpractice system, I agree that enterprise liability or "no fault" insurance would be a good place to start. An even better solution would be to develop a public health approach to reducing medical error to prevent avoidable harm to patients in the first place.

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Managed Care Insurance and Use of Higher-Mortality Hospitals

To the Editor: Dr Erickson and colleagues[1] concluded that patients with managed care insurance were less likely than those with fee-for-service (FFS) insurance to undergo coronary artery bypass grafting (CABG) at hospitals with lower mortality rates. However, the data in Table 1 of their article provide evidence that CABG inpatient mortality rates among patients enrolled in managed care plans are equal to or better than mortality rates among those in non–managed care plans. The authors minimize this central finding because they focus on the likelihood of using "lower-mortality hospitals," a very difficult metric to interpret.

Hospital use does not necessarily represent insurer contracting decisions. Indeed, Erickson et al did not determine which hospitals managed care organizations did or did not typically contract with. Rather, they contrasted hospital choices of physicians for very dissimilar patient populations. Given the lower mortality rates among individuals in managed care organizations compared with those with FFS insurance, it is apparent that the hospitals used for CABG were very effective for managed care patients but not as effective for patients with FFS insurance.

A survey of more than 2000 consumers conducted by the Kaiser Family Foundation and the Agency for Health Care Policy and Research found that a majority of consumers (72%) would choose a hospital that is familiar to them over a hospital rated higher in quality by experts.[2] The same survey concluded that an individual's regular physician has the most influence over the patients' choice of hospital. Patients choose local practitioners for convenience, and those practitioners have privileges at local hospitals.

Erickson et al did not consider several confounding variables for the classification of hospitals as high or low mortality. However, the demographic compositions of managed care and non–managed care populations were quite different based on the criteria used in the model. It is likely that additional confounding variables were overlooked. For example, Erickson et al acknowledge that patients in the study were more likely to choose a hospital they classified as a "higher-mortality hospital." In addition, the methods used to control for travel distance to hospitals were inadequate.

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1. Erickson LC, Torchiana DF, Schneider EC, Newburger JW, Hannan EL. The relationship between managed care insurance and use of lower-mortality hospitals for CABG surgery. *JAMA*. 2000;283:1976-1982.
2. Consumers value information on quality when selecting doctors and health plans. *Med Practice Communicator*. 1997;4:3.

To the Editor: In the study by Dr Erickson and colleagues,[1] the effect of clinical urgency on choice of hospitals was not sufficiently examined. The timing of CABG is closely correlated to clinical severity[2] and has a marked influence on the choice of hospital. In an emergency, patients are likely to be admitted to the facility nearest their place of residence. In terms of service provision management, clinical severity may, on the one hand, yield a priority rating (based on explicit criteria); on the other hand, it may determine the referral facility by taking into account bed availability and the fact that facility outcomes depend, among other things, on case mix severity and access procedure. For example, Showstack et al[3] showed that lower-volume hospitals only had higher adjusted mortality rates for nonscheduled patients, while there was no such correlation for scheduled patients.

In the study by Erickson et al, the absence of clinical data prevents assessment of this extremely important potential bias. Supposing that the administrative definitions "urgent" and "nonurgent" reported in Table 3 of their article truly reflect patient status, if only nonurgent cases are considered

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(because the treatment destination of urgent cases may have been greatly affected by distance from place of residence), then the relative risk of use of lower-mortality hospitals was exactly the same for private FFS and Medicare FFS insurance groups (relative risk = 1), while the relative risk for the privately managed care insurance group was 0.81. Thus, patients with less severe clinical status and scheduled CABG procedures are likely to have outcomes that are less variable and facility-dependent.

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To the Editor: Dr Erickson and colleagues[1] reported that New York State patients with private managed care insurance were less likely to undergo CABG surgery in hospitals with below average risk-adjusted mortality rates than patients with FFS insurance. Our analysis,[2] also based on data from the New York State hospital discharge database for 1996 but using somewhat different methods, suggests that the results of Erickson et al mask important regional differences. We believe that these differences are worth noting, not only with respect to specific conclusions, but also as a methodological caution about making inferences from models that pool together data from different regional markets.

Patterns of CABG surgery in New York State indicate that there are 9 distinct markets.[3] Patients generally choose hospitals and surgeons within their geographical region. The relevant choice set, therefore, is the set of hospitals or surgeons available in the region. Without variation in quality, it is not possible to statistically assess the factors that influence patients' quality of care. Table 4 in the article by Erickson et al shows that in some regions (Long Island and Rochester) all hospitals had either higher or lower mortality. Estimating a model that pools data across these regions incorrectly attributes the variation to factors, such as type of insurance, when, in fact, the variation is purely regional.

We discovered this problem in the course of our analysis investigating racial differences in access to high-quality cardiac surgeons. In that analysis, we used models with the risk-adjusted mortality rate of patients' CABG surgeons as the dependent variable.[2] A correctly specified model at the state level required not only indicators for regions, but interaction terms involving region and key covariates, such as type of insurance. In our analysis, managed care patients in 4 of the 7 regions analyzed (Albany, Buffalo, Syracuse, and Westchester) had, on average, surgeons with lower risk-adjusted mortality rates than patients with FSS insurance. In line with the findings of Erickson et al, 3 regions favored those with FFS insurance (Nassau, New York City, and Rochester). This is a much more complex picture than suggested by Erickson et al, and it highlights the importance of recognizing regional market variations in studies of health services.

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In Reply: Dr Ensor and colleagues misinterpret the mortality rates in Table 1 of our article, which are not adjusted for severity of illness or other preoperative risk factors. These raw mortality rates should not be used to compare outcomes of managed care and FFS-insured patients because the 2 groups of patients are known to differ. Also, while insurers are not the only participants in the choice of hospital, and patient convenience (particularly with respect to travel distance) is also an important factor,[1] it is difficult to imagine how this convenience factor would differ for patients in managed care plans and those with FFS insurance after controlling for travel distance (the "straight-line" distance method we used has been shown to correlate highly with actual travel times).[2] There is little reason to believe that patients with managed care insurance would have a great deal more difficulty with transportation to hospitals than patients with FFS insurance, particularly after controlling for relative affluence.

The risk-adjusted mortality rates published by the New York Department of Health[3] are among the most carefully defined and researched outcome measurement systems in the United States. They are based on a prospective clinical database, designed by a consortium of academic and government authorities in the field, and we know of no better data source for classifying hospitals on the basis of outcomes.

We agree with Dr Mariotto that urgent and nonurgent admissions need to be considered separately for the reasons he describes. We have assumed that the designation of urgency recorded by the admitting hospital correlates with the admittedly far more complex, almost certainly continuous, concept of clinical urgency. It is important to note, however, that for both urgent and nonurgent admissions, the patients with managed care insurance were less likely to be admitted to lower-mortality hospitals than were patients with FFS insurance.

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The issue raised by Dr Mukamel and colleagues underscores the complexity of health services research in general. Regional variations in referral patterns are likely to be common, since the characteristics of insurance plans, hospitals, patients, and referring physicians vary regionally. It would not be surprising if variations existed within the regions defined by Mukamel et al.[4] To address this confounding by region and type of confounding, we performed a sensitivity analysis, restricting the analysis to patients living within 25 miles of both a lower- and a higher-morality center. This analysis suggested that in regions with similar geographic access to both lower-mortality and higher-mortality centers, the discrepancy between managed care patients and those with FFS insurance was, in fact, even greater than in the state as a whole. The findings of Mukamel et al that managed care patients had lower risk-adjusted mortality in 4 of 7 regions they defined has to be considered in light of the fact that those 4 regions were substantially less populated than others (half of the state's population lives in New York City and Nassau County) and, therefore, have less impact on our findings.

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RESEARCH LETTERS

Garlic as an Insect Repellent

To the Editor: Lyme borreliosis is the most common vector-borne disease in Sweden, and as many as 10 000 individuals are thought to be affected each year.[1] Recent studies have suggested that individual variability in vector attachment may be linked to different body odors.[2,3] Other studies suggested that diethyltoluamide is the best repellent against insect vectors and permethrin against ticks, in particular. However, insect repellents may have adverse effects on humans and animals.[4]

Because military personnel are at particularly high risk for tick bites and tick-borne diseases,[5] we conducted a prospective, randomized, double-blind intervention trial of garlic (*Allium sativum*) to prevent tick bites among Swedish marines.

Methods. Of 100 individuals in Swedish military service in 1998, 50 consumed 1200 mg/d *Allium sativum* in capsule form and 50 consumed placebo for 8 weeks, followed by a washout period of 2 weeks, and then a crossover to placebo or *Allium sativum* consumption for another 10 weeks. All participants wore the same type of uniforms, consumed approximately the same diet, participated in similar activities, and spent equal amounts of time in tick-endemic areas. Tick bites were recorded in a diary after daily self-inspection of the skin. Written informed consent was obtained from all participants. The study was approved by the Research Ethics Committee of Lund University and the Swedish Medical Products Agency. Data were analyzed by both intention to treat (involving all participants present at the start of the study) and per protocol (only the 80 individuals who completed the study).

Results. In the intention-to-treat analysis, 66 (66%) of 100 participants recorded tick bites vs 55 (69%) of 80 participants in the per-protocol analysis. A total of 286 tick bites were recorded by the participants. On average, the participants recorded 0.2 tick bites per week during military service, compared with 0.03 tick bites during leave. There was significant reduction in tick bites when consuming garlic compared with placebo in per protocol analysis (Wilcoxon test, $P=.04$). A greater number of the participants were bitten by ticks during placebo consumption (normal approximation of binomial assumption, relative risk by intention to treat, 0.79 [95% confidence interval {CI}, 0.65-0.96]; relative risk per protocol, 0.70 [95% CI, 0.54-0.90]).

Comment. Swedish marine conscripts are at high risk of tick bites during military service. Preventive measures, including vaccinations against tick-transmitted diseases, should be considered. However, our results suggest that garlic may be considered as a tick repellent for individuals and populations at high risk for tick bite, rather than other agents that might have more adverse effects.

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Evidence of Myocardial Infarction in Mummified Human Tissue

To the Editor: Pathological study of mummified soft tissue may help detect epidemiological patterns of disease in ancient populations.[1] We hypothesized that by using a sensitive and specific cardiac troponin (cardiac troponin I [cTnI]) assay,[2] we could detect molecular evidence of a myocardial infarction (MI) in the mummified tissue of an individual in whom other forensic

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and historical evidence suggested sudden cardiac death. Such a demonstration would allow case studies of heart disease in historical populations, similar to ongoing investigations into ancient patterns of parasitic disease.[1]

Horemkenesi was a foreman of craftsmen excavating and decorating the tombs of the pharaohs of the 20th dynasty (c 1050 BC) at Thebes,[3] where he supervised workmen camping on the desert plateau during their 10-day shifts. He was also a priest of Amun, and his responsibilities required frequent journeys of several miles in the desert. Forensic study of his mummy indicates that at about age 60 years he fell face downward in the sand and was heavily infested with carrion beetles before mummification, suggesting sudden cardiac death.[3] After desiccating in sand, Horemkenesi's body was apparently further desiccated in natron, a mixture of sodium carbonate and sodium bicarbonate that was often used to prevent putrefaction.

We tested the mummified abdominal tissue of Horemkenesi for the presence of cTnI, which would be consistent with death from acute MI. We also measured cTnI in mummified tissue from modern subjects who had died of acute MI and other causes. This allowed us to assess the stability of cTnI to dessication and its specificity for acute MI in mummified tissue.

Methods. Desiccated tissues from Horemkenesi's abdomen were extracted for myofibrillar proteins in a 50 mmol/L Tris–8 mol/L urea buffer, dialyzed against 10 mmol/L Tris, pH 8.0, at 4°C for 18 to 24 hours, frozen, lyophilized, and reconstituted with cTnI-negative human sera. Modern positive controls (spleens from subjects who died of acute MI) and negative controls (tissues from subjects who died of other causes) were mummified by allowing them to desiccate at room temperature, either in sand, natron, or a desiccator, for 40 days. The level of cTnI was then measured in an identical fashion to the measurement done for Horemkenesi's tissue.

Results. Two samples of abdominal tissue of Horemkenesi yielded 12.3 ng/g and 22.2 ng/g of cTnI per tissue weight. Mummified positive controls demonstrated cTnI in 2 of 2 specimens: 1.3 ng/g in spleen dried in sand and 3.7 ng/g in spleen desiccated in natron. Mummified negative controls demonstrated measurable cTnI values in 3 of 6 tissues tested: 0.7 ng/g in a spleen dried in sand, 0.9 ng/g in a spleen desiccated in natron, and 0.8 ng/g in skeletal muscle dried in a desiccator.

Comment. Our preliminary findings demonstrate that cTnI is preserved through time and that cTnI levels are higher in desiccated tissue of subjects who died of acute MI. The higher concentration of cTnI following desiccation in natron suggests that more rapid desiccation with natron resulted in better preservation of diagnostic epitopes.[4]

Although our data suggest that myocardial death can be diagnosed several thousand years after the event, these findings should be seen as preliminary. First, other tissues from this mummy, as well as those with similar histories, should be assessed for the presence of cTnI. Second, modern controls need to be developed that more closely resemble ancient mummified tissues. Such research could help estimate the prevalence of deaths from acute MI in historical populations when soft tissue has been preserved.

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Cerebral Hemorrhage Following a Roller Coaster Ride

To the Editor: Previous reports[1-5] suggest that neurological injury following a roller coaster ride may occur among elderly persons, those with known malformations within the brain and spinal cord, and those receiving anticoagulant medications. We report a case of multiple cerebral contusions and subarachnoid hemorrhage occurring in a healthy person without known risk factors for these injuries following a roller coaster ride.

Report of a Case. A healthy 47-year-old male physician experienced a transient episode of severe nausea and dysequilibrium immediately after a ride on a high-speed roller coaster, in which he had been violently swung in several directions. There was no history of head injury, either before or after this incident. Seven days later he experienced a sudden onset of diffuse headache, nausea, and vomiting. After the fourth consecutive day of these symptoms he was seen in our hospital, where he presented with meningismus, but was without focal neurological signs.

He was found to have normal clotting parameters. However, a computed tomographic scan of the brain revealed small hemorrhagic lesions in both the right frontal pole and the left temporal cortex, which were confirmed by magnetic resonance imaging (FIGURE). Cerebrospinal fluid was xanthochromic and was found to have a high bilirubin concentra-

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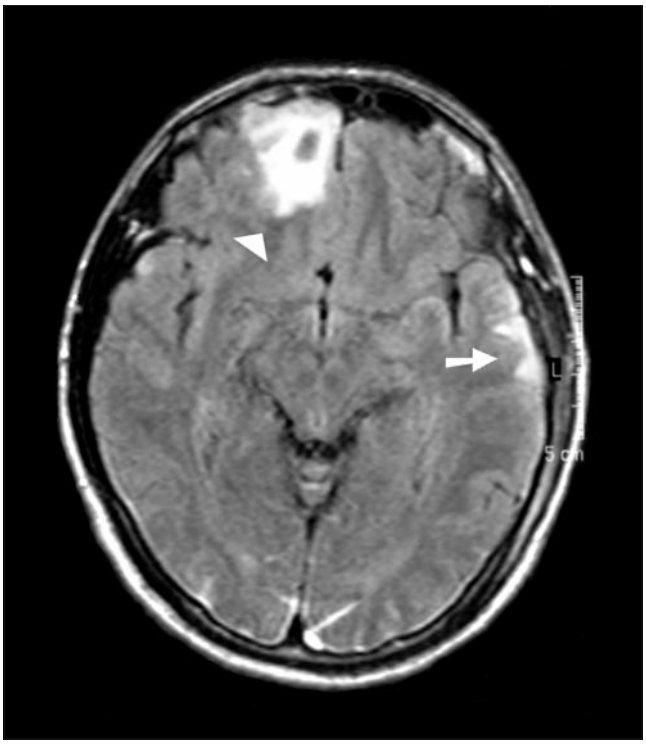
Figure. Magnetic Resonance Image of the Brain



Hypointense lesion surrounded by hyperintensity (arrowhead) in the right frontal pole and hyperintense lesion in the left temporal cortex (arrow) represent areas of focal hemorrhage.

tion, suggesting recent subarachnoid bleeding. Total cerebrospinal protein and nucleated cells were within the normal range. A digital subtraction angiogram did not reveal any aneurysms, arteriovenous malformations, or other vascular anomalies. The patient gradually recovered, and a week later, at discharge, he complained only of mild headache.

Comment. This is the first reported case, to our knowledge, of multiple cerebral contusions and subarachnoid hemorrhage occurring in a healthy person following a roller coaster ride. We are confident, on the basis of the patient's history, that he did not experience any further head trauma in the 7 days between the ride and the onset of his symptoms. This prolonged interval might be explained either by secondary subarachnoid bleeding from asymptomatic intracerebral contusions, or by delayed posttraumatic hemorrhage.

This case underlines the importance of obtaining a thorough history in the event of an otherwise unexplained intracerebral or subarachnoid hemorrhage. Although the incidence of brain injury induced by a roller coaster ride is presumably low, it might become more common with the introduction of faster and more complex rides. Riders of roller coasters should be warned about the risk of brain injuries, and improved safety features for their prevention should be considered.

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CORRECTION

Incorrect Name in Reference: In the Commentary titled "Sustaining Change in Medical Education" published in the May 10, 2000, issue of THE JOURNAL (2000; 283:2429-2431), on page 2431 reference 10 contained an incorrect name. Instead of Joyce A. Friedland the presenter's correct name is Joan A. Friedland (the reference should have read "Friedland JA").

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Suffolk County Vector Control & Wetlands Management Long Term Plan & Environmental Impact Statement

Steve Levy, County Executive

Suffolk County Vector Control and Wetlands Management Revised Long-Term Plan

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October 2006

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SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT

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This plan was prepared by Cashin Associates, PC, with assistance from Cameron Engineering & Associates, LLP. Review was provided by Wayne Crans, PhD (Rutgers), Suffolk County Vector Control (Dominick Ninivaggi, Supervisor) and Suffolk County Department of Health (Division of Environmental Quality, Vito Minei, PE, Director, Walter Dawydiak, PE, Chief Engineer, and Division of Public Health, David Graham, MD, Director, Scott Campbell, PhD, Arthropod-borne Disease Laboratory Director). It was revised in response to public comments received following its initial release in October, 2005 by Cashin Associates, PC. It was further revised following the receipt of comments on the May 3, 2006 release of the Long-Term Plan and its associated Draft Generic Environmental Impact Statement by Cashin Associates.

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APPENDICES

Appendix 1. Pesticide Labeling Requirements

The Revised Wetlands Management Plan (October 2006) is appended by reference.

LIST OF ABBREVIATIONS AND ACRONYMS

ABDL	Arthropod-Borne Disease Laboratory
Ach	Acetylocholine
AChE	Acetylcholinesterase
AMCA	American Mosquito Control Association
BMPs	Best Management Practices
BOCES	Suffolk County Board of Cooperative Educational Services
CCMP	Comprehensive Conservation and Management Plan
CDC	Centers for Disease Control and Prevention
DDT	Dichloro-Diphenyl-Trichloroethane
EEE	Eastern Equine Encephalitis
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FINS	Fire Island National Seashore
GIS	Geographic Information Systems
IMM	Integrated Marsh Management
IPM	Integrated Pest Management
MCC	Mosquito Control Commission
NYSDEC	New York State Department of Environmental Conservation
NYSDOH	New York State Department of Health
MEDFLY	Mediterranean Fruit Fly
OMWM	Open Marsh Water Management Plan
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PHL	Public Health Law
RAMP	Rapid Analyte Measurement Platform
SCC	Suffolk County Charter
SCDEE	Suffolk County Department of Environment and Energy
SCDHS	Suffolk County Department of Health Services
SCDPW	Suffolk County Department of Public Works
SCVC	Suffolk County Vector Control
SEQRA	State Environmental Quality Review Act
ULV	Ultra Low Volume
USEPA	United States Environmental Protection Agency
VCMS	Vector Control Management System
WHO	World Health Organization
WNV	West Nile Virus

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ABSTRACT

Suffolk County has developed this Long-Term Plan to control mosquitoes and enhance wetlands. A major goal is to reduce larviciding by 75 percent, as measured in acres treated, over 12 years; currently, 4,000 acres of tidal wetlands are routinely larvicided. Another key goal is to continue to reduce adulticiding. In recent years, less than two percent of Suffolk County has received non-emergency adulticide treatments.

The Plan will enhance integrated pest management, including increased surveillance (including pre-adulticide, and post-adulticide efficacy), operational improvements (e.g., catch basin larviciding), and expanded public education/outreach. Strict numeric mosquito criteria will be used to justify every non-Health Emergency adulticide treatment. The use of technology has also been optimized. For example, the Adapco Wingman spray technology is used to minimize pesticide usage, and geographic information systems have been improved.

Wetlands management will be critical in reducing larvicide usage. As part of the program, no new ditches will be created, and routine use of machine ditch maintenance has ceased. During the first three years the program will focus on low-impact water management, such as replacing culverts restoring tidal circulation and improvement to fish habitats without significant changes to the wetland. Wetlands functions and values will be the paramount objective for all projects. In the longer term, a Wetlands Stewardship Committee strategy will address the assessment and management needs of all 17,000 acres of tidal wetlands in Suffolk.

The Long-Term Plan was developed based on a series of extensive and unprecedented field initiatives, including 21 detailed marsh evaluations and a major Integrated Marsh Management demonstration at the Wertheim National Wildlife Refuge. The Plan also included tests of mosquito control alternatives, studies of stormwater and benthic invertebrates, and analysis for pesticides in hundreds of samples (part-per-trillion level).

The Long-Term Plan was supported by a comprehensive risk assessment which determined that selected vector control agents (e.g., Bti, methoprene, resmethrin, and sumithrin) pose negligible human health risks, and result in no significant ecological impacts. Modeling suggested, that, in the absence of vector control, tens of West Nile virus deaths and hundreds of serious illnesses might occur in Suffolk County each year; currently, illness rates are much less.

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EXECUTIVE SUMMARY

This Long-Term Plan's primary goals are to decrease potential risks to human health and public welfare from mosquitoes and mosquito control measures, and to reduce the use of pesticides for vector control. An ambitious target of a 75 percent reduction in larvicide usage (as measured in the number of acres treated) has been set. The area treated with adulticides has already been reduced by more than 50 percent in the past five years, and this Long-Term Plan seeks to further reduce use of adulticides.

The Long-Term Plan also seeks to use a series of Best Management Practices (BMPs) to restore approximately 4,000 acres of tidal wetlands that were grid ditched in the 1930s, and which now require routine larvicide applications by air. When implemented, the BMPs will reduce or eliminate the need for larvicide on these lands, and improve wetland values by enhancing biodiversity and limiting invasive species, including *Phragmites*. Wetland restoration strategies will be tailored to the specific needs of individual marshes with mosquito control as one element considered in the overall restoration strategy. The greatest reductions in larvicide use can be achieved by prioritizing project sites where mosquito production is greatest, the most pesticide is used, and relatively simple measures will reduce or eliminate larval habitat. Once a site is chosen, however, the design process should consider the preservation and/or enhancement of natural resource values as the most important factor in choosing which BMPs will be used for mosquito control.

Among the significant policy commitments contained in the Long-Term Plan are:

- The continuation of the "no new ditching" policy, and establishment of a presumptive interim policy of ditch reversion as opposed to ditch maintenance. It is expected that less than 50 acres of salt marsh per year will be subject to machine ditch maintenance, and then only when necessary to address a critical ecological or public health need (e.g., to restore tidal circulation or to eliminate a severe infestation).

- Institution of a framework for continuing coordination and input by the Citizen and Technical Advisory Committee structure to help guide the preparation of Triennial Plan updates.

- The creation of a Wetlands Stewardship Committee comprised of agencies, policymakers, estuary program representatives, and non-profit institutions. The Stewardship Committee will receive early notice of all potentially significant wetlands management projects (except for the most minor initiatives, such as maintenance of culverts). The Stewardship Committee will also approve all major wetlands restoration proposals, with the overarching goal of enhancing salt marsh functions and values. Building on accomplishments of the Wetlands Management Plan, the Committee will also be charged with developing a strategy to begin addressing the management needs of all of the County's 17,000 acres of tidal wetlands, irrespective of Vector Control significance. This process will include refinement of preliminary wetland health indicators described in the Wetlands Management Plan.

Critical Long-Term Plan recommendations include the continued use and refinement of integrated pest management (IPM) procedures, and improvements in surveillance (e.g., better documentation of mosquito populations and post-spray efficacy, and the establishment of additional mosquito traps at Fire Island National Seashore locations). In order to improve source control at breeding sites, the Long-Term Plan calls for enhanced catch basin larviciding. Expansion of public education and outreach is highlighted through improved used-tire management, greater use of providing information through Internet contact, and promoting source control methods at businesses and homes.

Early action projects are a hallmark of the Long-Term Plan. Examples include implementation of a new technology to guide pesticide applications. The "Adapco Wingman" system uses a computer model and real-time meteorological data to minimize pesticide usage and to optimize mosquito control.

In another early action, a progressive Integrated Marsh Management (IMM) project restored 80 acres of grid-ditched salt marsh at the Wertheim National Wildlife Refuge, thereby controlling mosquito breeding and enhancing wetland values such as biodiversity. IMM improves habitat for wildlife, native vegetation and larvae-eating fish by creating ponds for aquatic habitat channels for proper tidal circulation, eliminate and filling obsolete ditches that altered marsh hydrology. At the same time, IMM controls mosquito production by a combination of fish

predation and elimination of stagnant pools where mosquito predators can not survive. The project was conducted in cooperation with the US Fish and Wildlife Service, and is the first of its kind on Long Island. Supported by extensive monitoring, this initiative will serve as a test of the viability of future IMM projects on Long Island.

Tidal wetlands restoration strategies will proceed in accordance with three-year work plans. With the possible exception of Wertheim, no new major IMM projects are expected during the 2007-2009 timeframe. Future IMM recommendations will be subject to Wetlands Stewardship Committee approval, as part of the Triennial Plan update process.

The scope of this Long-Term Plan addresses wetlands that are subject to Vector Control operations. Approximately 4,000 acres of grid-ditched salt marsh are proposed for "reversion" via natural processes, i.e., no management for vector control is needed. As previously stated, another 4,000 acres of wetlands which are routinely larvicided will be evaluated for restoration via minor Best Management Practices (BMPs) (e.g., maintain/repair existing culverts) or, in the long-term, major restoration (subject to Stewardship Committee review). The county's remaining 9,000 acres of wetlands require additional assessment, and any major restoration projects proposed will be subject to SEQRA review. Remote sensing is expected to allow for cost-effective monitoring of the County's wetlands and supplement field visits.

It is envisioned that the process of assessment and enhanced wetland management be completed within 12 years. For individual projects, wetlands management goals for mosquito control must be adapted to the management goals set by landowners and natural resource managers, and may not be driven by vector control considerations. The ability to successfully implement Long-Term Plan objectives will be dependent upon cooperation by many agencies and stakeholders.

The Long-Term Plan will continue to be a cooperative effort administered by Suffolk County Department of Health Services (SCDHS), in cooperation with Suffolk County Department of Public Works (SCDPW). The Suffolk County Department of Environment and Energy (SCDEE) will be a lead partner. Suffolk County has already budgeted several new staff positions to begin implementation of the Long-Term Plan's recommendations although most can begin to be accomplished with existing resources. Grant programs and supplemental funding sources will be sought, in particular for wetland restoration projects.

Impetus for Long-Term Plan

Suffolk County sponsored this comprehensive evaluation of its Vector Control program to develop strategies to best protect public health, while optimizing environmental quality. Reasons for initiating the Long-Term Plan included:

- The West Nile virus threat, intermittent reappearance of Eastern Equine Encephalitis, and other vector-borne diseases, e.g., malaria. Suffolk County has had four deaths and 25 severe neurological illnesses attributable to West Nile virus between 1999 and 2006. Nation-wide, deaths total 891 through 2004, with over 7,000 severe neurological cases, with an average of 1,212 severe cases and 109 fatalities in the years 2004-2006.

- A long-standing need to better manage the legacy of grid-ditched wetlands to optimize environmental quality and reduce pesticide usage. By the end of the 1930's, over 90 percent of the County's 17,000 acres of salt marsh were grid-ditched for mosquito control purposes. The ditch network is substantially intact, but over 4,000 acres of marsh still require routine larvicide applications.

Background - Plan Approach

The Long-Term Plan followed a classic management plan approach rather than just evaluate impacts of a pre-determined outcome, i.e., a Generic Environmental Impact Statement on a pre-specified plan. It was based on data collection, evaluation of alternatives, and quantitative health and ecological risk assessments. The process was transparent, with extensive involvement by both Technical and Citizens Advisory Committees.

A comprehensive literature review was undertaken to determine the state-of-the-art in the fields of mosquito control, disease transmission, toxicology, wetlands biology, marine ecology, and environmental chemistry. Critical information was collected on mosquito biology and diseases, innovative mosquito control practices, mosquito control pesticides including their application technologies, formulations, and potential impacts, and wetlands and salt marshes.

Extensive local information was also collected and organized. This process included establishing a mosquito control-oriented Geographical Information System (GIS), digitized

mapping of the County's wetlands, and analyzing past and current mosquito control practices in the County. To support the analyses of potential impacts, four sections of the County were exhaustively described in terms of human use and ecological values, and 21 marshes were comprehensively studied.

Scientific studies and demonstration projects were conducted. Hundreds of samples were taken from air, water, sediment, and biota, and pesticides were measured to research level accuracy, i.e., one part-per-trillion. Mosquito control effectiveness of garlic oil, rosemary, and mosquito traps was tested. None of these "alternative techniques" showed promise for the County vector control program. Other studies were performed on benthic invertebrates, salt marshes with various larviciding histories, and stormwater in relation to ditches; vector control impacts were not found. Catch basins were evaluated, and documented to be problem mosquito breeding sites.

Health or Nuisance?

One of the goals of the Long-Term Plan, which addresses human health and public welfare, was to evaluate the possibility of differentiating "health-based" vector control from another commonly used term: "nuisance" control. A true distinction proved to be impossible because all the major mosquito species found in Suffolk County that bite people are capable of spreading disease, and therefore, the public health risks from biting mosquitoes can never be said to be zero. Control prior to the actual detection of pathogens can also reduce the need for, and mitigate risks in, emergency response situations. Finally, health concerns from mosquito infestations exist (pain, itching, possibility of infections, etc.), irrespective of detected pathogens. Vector control clearly results in ancillary quality-of-life benefits, but this is not the primary reason for a mosquito control program. The Long-Term Plan thus approaches mosquito control in terms of the continuum from "vector control" (e.g., low but present disease and health concerns) to "public health emergency" (e.g., pathogen response in accordance with federal and state guidance). "Vector control," in this context, can be used synonymously with "*public health* nuisance control." Indeed, the Public Health Law and the New York State Department of Health utilize the "public health nuisance" model to characterize mosquito infestations in the absence of a detected pathogen.

For vector control scenarios, strict numeric criteria for adulticiding have been adopted which require that quantitative mosquito thresholds be met prior to any adulticide application. Vector Control will keep records to document all pesticide application decisions. Emergency response actions will be conducted in conformance with West Nile virus response guidelines.

Vector Control Agents: Results of Risk Assessment

The Long-Term Plan evaluated risks to public health associated with vector control alternatives from vector-borne diseases as well as exposure to pesticides, and weighed these risks against potential impacts to the environment. The approach is especially noteworthy in that it addresses physical, chemical, and biological stressors. The public health risk assessment determined that, in the absence of any vector control, Suffolk County could expect to see as many as 16 deaths from West Nile Virus each year, and 150 persons could contract serious West Nile illness. Impacts from EEE and other diseases could not be quantified, but the threats are grave.

The exhaustive toxicological (pesticide) risk assessment was based on extremely conservative, worst-case assumptions and showed negligible human health impacts and minimal ecological impacts (i.e., not significant). The results for Vector Control agents are summarized as follows:

- Human health: negligible impacts (acute, chronic, or carcinogenic) from any larvicide or adulticide agent.

- Ecological impact: no impacts for mammalian, avian, or reptilian wildlife from any pesticide. Possible aquatic impacts were associated only with the adulticides permethrin, and potentially more so from malathion. However, the invertebrate impacts do not propagate up the food chain, and the model showed recovery to be complete by the following spring.

Bees are the standard for understanding agricultural pesticide impacts to flying insects and, based on theoretical potential effects to bees, all adulticides posed a potential risk to non-target flying insects. However, vector control adulticides are generally not applied when bees are flying (day time). No study has attributed significant impacts to insect populations from vector control adulticides at the concentrations and methods in which they are applied. Also, the literature suggests that effects of transient stressors on insect populations are fleeting, with populations recovering within days.

Part of the effort to develop the Long-Term Plan was to evaluate typical risk from the use of pesticides in everyday life. The exposures and corresponding human health risks from the use of pesticides for vector control purposes are small relative to other risks, such as those associated with exposure to pesticides in food, indoor residential use and some pet flea and tick products. The insect repellent DEET was also evaluated. Proper use of DEET products should not result in adverse health impacts.

An extensive "Caged Fish" study found no lethal or sublethal impacts to organisms attributable to applications of resmethrin and methoprene. In fact, researchers found that the pesticides actually decayed more rapidly in the environment than prior laboratory based studies suggested.

Conclusion

It is the policy of Suffolk County that pesticides should always be used sparingly, and only when needed. This study has demonstrated that the benefits of carefully controlled Vector Control program, conducted within an Integrated Pest Management framework, clearly outweigh the potential adverse impacts, which have not been found to be significant and which are mitigated by the IPM measures described in the Long-Term Plan. Moreover, marsh restoration can have a significant positive environmental impact, while controlling vectors and reducing or eliminating the need for pesticide usage. Therefore, implementation of the Long-Term Plan should achieve its major goals of reducing impacts to human health while significantly improving overall County ecological conditions.

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OVERVIEW OF THE PLAN

Introduction

Mosquitoes impact human health and public welfare in Suffolk County. To alleviate these impacts, the Suffolk County Charter established a vector control agency, and charged it (in concert with the Department of Health Services) to protect the citizens of the County from disease and other deleterious effects of mosquito infestations.

This Long-Term Plan uses the Integrated Pest Management (IPM) approach to mosquito management to decrease risks to human health and impacts to public welfare from mosquitoes and mosquito management. Simultaneously, implementation of the Long-Term Plan will reduce impacts to the environment and provide potential ecological benefits. The Long-Term Plan stresses public education and associated source reduction as key elements to achieve these goals, with progressive marsh management implementation being necessary to achieve significant reductions in overall pesticide use. The Long-Term Plan also relies on scientific surveillance of mosquito conditions to target mosquito problems that may remain through use of biorational larvicides. If adult mosquito populations of concern still exist, and rigorous decision criteria are met, adult mosquito control with pesticides may be employed.

Progressive Water Management

As part of the Long-Term Plan development process, the County was able to implement a progressive water management demonstration project at Wertheim National Wildlife Refuge, the first of its kind on Long Island. This wetlands restoration project appears to have created a more diverse salt marsh that enhances wildlife and finfish habitat values, using Open Marsh Water Management (OMWM) techniques (which achieve mosquito management by improving habitat for fish to consume mosquito larvae). This is an alternative to maintenance of the legacy grid ditch system for mosquito control, and, as implemented elsewhere in the northeast US, has led to significant reductions in the acreage and instances of pesticide usage. Similar kinds of water management projects in other jurisdictions have also been found to provide significant improvements to the ecological functions of treated marshes, especially improving habitat for marsh birds and nekton.

Salt marshes are not the sole source of mosquitoes in Suffolk County, but the species that do develop there cause public health and welfare impacts. The Long-Term Plan proposes serious consideration of the implementation of a selection from 15 progressive water management Best Management Practices across 4,000 acres of salt marsh, areas the County identified as probably needing restoration because they are currently larvicided regularly to control mosquito populations. Implementation of progressive water management at all of these sites might be expected to result in the elimination of up to 75 percent of current larvicide use (as measured by acres of marsh treated in a year, in comparison to a baseline of 30,000 acres).

These assumptions are supported by decades of experience at other northeast US salt marshes. So far they have been validated by monitoring at the Wertheim site. Progressive water management is expected to serve as the basis for a vector control program that relies on natural processes where control is necessary to address mosquito problems, and therefore one that will be able to substantially reduce pesticide applications.

However, concerns raised by a broad spectrum of involved parties have persuaded the County to slow implementation of its Wetlands Management Plan. The County will create a Wetlands Stewardship Committee to create a broad definition of salt marsh health. The Committee will have a membership drawn from governmental agencies at all levels, but will also include local representatives and non-governmental, environmental advocacy organizations. This definition of marsh health will be used to develop a comprehensive marsh management plan that includes factors other than mosquito management. The Integrated Marsh Management program that will be developed from the plan will include mosquito control projects, but in the overall context of comprehensive coastal marsh planning and marsh health.

The Wetlands Stewardship Committee will have broad review responsibility for proposals using those water management projects with activities that may cause the greatest alterations to existing marsh conditions, and for other projects of interest or concern to the Committee membership. In addition, for all but the most basic projects, the County will require that water management projects undergo additional State Environmental Quality Review Act (SEQRA) review, as recommended by the Wetlands Stewardship Committee, and as developed by the Council on Environmental Quality.

Nonetheless, this tidal wetlands management approach represents a dramatic change in County direction, from a decades-long commitment to ditch maintenance to a general presumption in favor of marsh reversion as an interim policy. Some 4,000 acres of County salt marsh are slated for non-intervention for vector control purposes, although it is possible that wetlands in this category may require active management to restore necessary ecological functions, as developed by the Integrated Marsh management program. An additional 4,000 acres have been identified as high priority sites for progressive water management to address mosquito control needs. The remaining 9,000 acres of County salt marsh will be assessed over the next 10 years, with appropriate management actions developed cooperatively by all interested parties in light of the assessments, and in the service of the broad County Integrated marsh management program for its salt marsh resource. While this program is developed, it is expected that ditch maintenance (when essential for public health or ecological reasons) will affect less than 50 acres of tidal wetlands per year. The pre-existing policy of "no new ditching" (except when necessary for restoration) will, of course, continue to be in effect, and any essential ditch maintenance will be conducted in accordance with criteria described in the Long-Term Plan.

Implementation of the Wetlands Management Plan, as modified by the development of the Integrated Marsh management program, not only can reduce pesticide usage and provide a sound basis for salt marsh mosquito management, but will serve as a basis for managing the marshes themselves in a healthier, more sustainable manner. Salt marshes have been identified as key features in the County's shoreline ecosystems, and clearly play a role in regulating water quality and sustaining estuarine food chains. Improving their overall health is a clear necessity for any overall restoration plan for the County's coastal environment.

Scientifically-based Decision Making

All marsh and mosquito management decisions will be based on scientifically-grounded data generation. The County currently has one of the most comprehensive surveillance programs for mosquito monitoring. This network this will be expanded, which will allow the County:

- to determine exactly where and how to reduce larval populations of mosquitoes to prevent mosquito problems from occurring;

- to look for the presence of and then further track the progress of mosquito-borne pathogens that can threaten human health;
- to prevent untoward impacts to public welfare from aggressive human-biting mosquito populations that also threaten to transmit disease; and,
- to carefully and specifically target those mosquitoes that require treatment as adults to prevent impacts to human health, in accord with State and federal guidelines.

Surveillance activities have been the basis for mosquito management decision-making. The Long-Term Plan, by augmenting existing networks, and establishing new means of testing mosquito populations and the effectiveness of its operations, will improve the County's ability to make crucial decisions based on best available information.

In addition, in a new approach to public outreach, the County will use these data to clearly and concisely explain to the public why it made particular treatments (*i.e.,* to document pre – treatment mosquito levels in relation to criteria which trigger treatment) and to demonstrate the efficacy of the control efforts. This will help the general public understand why particular actions were taken when they were, how effective they were, and to potentially appreciate the benefits received from progressive vector control.

Plan Approach

The Long-Term Plan follows the tenets of IPM. It is composed of 11 parts.

The first sets out the goals and objectives of the Long-Term Plan, identifies the mosquitoes found in the County, which ones represent potential problems for people, and gives the basis for actions by Suffolk County Vector Control to address any mosquito problems that may arise. It demonstrates the County's commitment through its vector control activities to decrease impacts to human health and public welfare, and to decrease environmental impacts while also restoring significant portions of the County's environment.

The second part of the Long-Term Plan focuses on public education and outreach, which, when successful, can eliminate the need for organized control activities. Public education focuses on two main objectives:

- ensure that residents take personal responsibility, as much as is possible, for deterring mosquito bites by avoiding mosquitoes, and using effective repellents as directed by the label when encountering mosquitoes;
- promote proper housekeeping, especially regarding standing water in the vicinity of homes and businesses, to deny pestilential mosquitoes the breeding habitats they need.

The third part of the Long-Term Plan addresses surveillance activities, which generate the data that allow for scientifically-grounded treatment actions. Surveillance defines where the mosquitoes are, and what diseases they may be infected with. The risk that they will impact the health and welfare of the citizens of the County can then be determined. The County is expanding its surveillance activities, and adapting them to face new issues, such as other emerging diseases and changes in the ways existing disease can be tracked.

The fourth part of the Long-Term Plan is source reduction, which is the most efficient means of addressing mosquito problems. Important programmatic elements include site inspections by Vector Control personnel, discarded tire management, and maintenance and upkeep of storm water systems. For example, proper efforts in these aspects of source reduction lead to decreased breeding opportunities for some species that are essential for West Nile virus propagation.

Source reduction also includes water management, and the stand-alone Wetlands Management Plan has been appended to and made part of the Long-Term Plan. Water management is meant to be the centerpiece of the County's mosquito management efforts. Through water management, areas that are the source of the most aggressive, and, by some accounts, most dangerous mosquitoes in the County, can be largely reduced. Progressive water management holds the promise of not only effectively controlling mosquitoes, but serving as a means of enhancing natural marsh health. Implementation of the Wetlands Management Plan, in accord with an overarching Integrated Marsh Management program developed through the Wetlands

Stewardship Committee, should improve biodiversity and concomitant marsh values, while also achieving vector control aims, with little need for ongoing maintenance.

Biocontrols are also discussed in the Long-Term Plan. Inherent in progressive marsh management is the use of fish to control larval populations (thus reducing adult populations). The undeniable success of this biological control has raised many hopes that other, natural elements of the local ecosystem can be augmented or supported to provide consistent control of mosquitoes, as well. The evaluation of other mosquito predators showed that it was extremely unlikely that any can be implemented to achieve the degree of control necessary to reduce impacts from mosquitoes to human health and public welfare. Some fish and aquatic invertebrate species may be effective at larval control if released to carefully selected environments. The County will continue to educate itself regarding potential benefits from organisms that will prey on mosquitoes, and determine under what conditions they can be released to achieve acceptable control. Although some organisms clearly prey on adult mosquitoes under some conditions, it is difficult to increase naturally occurring populations so as to achieve good levels of control.

Pesticides are the most visible component of nearly all mosquito control programs, although mosquito control experts have long advocated an integrated approach to control. Pesticides have an important role in controlling populations of mosquitoes and the disease threat they represent, especially in a jurisdiction such as Suffolk County. Suffolk County has so many larval habitats that eliminating them all is a practical impossibility. Because many larval habitats are found in protected wetlands with important environmental values, eliminating many of these habitats is generally not desirable. Therefore, pesticides will be needed to provide acceptable levels of control. Part 6 of the Long-Term Plan addresses larval control, which, because the organisms are concentrated in aquatic environments, is more efficient than control of adult mosquitoes. Part 7 of the Long-Term Plan discusses the procedures for identifying when adult mosquito control might be considered, and, if extensive criteria for applying pesticides are met, exactly how those applications might be made (see Overview Table 1, below). The decision making process described here is consistent with and is based on guidelines published by State and Federal health agencies.

Overview Table 1. Adulticide Decision Parameters

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Basic Surveillance Parameters	Number of mosquitoes	Yes	No	Counts in light traps significantly above criteria; landing rates; complaints	Based on female, human-biting species; 25+ per NJ trap, 100+ per CDC trap; landing rate 1+/min.; complaints invaluable where traps are not set; intend to set CDC traps before all non-Fire Island applications
	Species present	Yes	Yes	Light trap content analysis	Information on basic mosquito biology essential: Vector Control targets aggressive biters; Health Emergency targets specific (bridge) vectors; ; intend to set CDC traps before all non-Fire Island applications
	Complaints	Yes	Yes	Number/location of calls	Evaluate in historic context; complaints must be supported with appropriate surveillance data; complaints document extent of problem better than traps can
	Historical population trends	Yes	No	Surveillance data records	Data patterns often signal that problem is about to abate, or is likely to worsen
Species Specific Parameters	Aggressiveness of target species	Yes	Yes	Documented biting patterns of trapped mosquitoes	Aggressive biters indicate greater problem, increased likelihood for bridge vector participation
	Activity patterns of target species	Yes	Yes	Documented host seeking patterns, flight ranges of trapped mosquitoes	Guides actual control decision; e.g., evening vs. later at night; day-time flying may inhibit control; spot treatments only effective for short flight range species; large flight ranges require applications to cover larger, continuous areas to be effective
	Vector Potential	No	Yes	Infection rate, vector competence, % mammalian meals of trapped species	Establishes relative risk for species present
	CDC Vector Index	No	Maybe	MIR, trap counts for all potential vectors	CDC light trap counts * MIR, summed over all vector species; higher index correlates to more human infections following week; requires high mosquito/human infection rates for use; can use only with multiple trap data sets

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Species specific parameters, continued	Parity rates	Sometimes	Yes	Age (blood meal history) of biting population	For Health Emergency, high parity rates indicate majority of biters had prior blood meal – direct indication of increased Vector Potential; for Vector Control, an aging population, even if smaller, will be treated since it represents increasing vector potential
	Life Cycle Type	Yes	Yes	Trap analysis	Brooded mosquitoes eventually die off on own, continuous breeders build populations over season
Public Health Parameters	Bird testing	No	Yes	Presence/absence of virus	Provides early warning in terms of bird to bird transmission; documents active disease foci in County
	CDC mosquito pool testing	No	Yes	Presence/absence of virus	Amplification vectors provide early warning, document active disease foci in County; bridge vectors indicate virus present in human-biting species, is signal that human health risk is imminent
	Veterinarian reports	No	Yes	Ill/dead target animals	Non-mammals provide early warning, document active disease foci in County; mammalian cases indicate virus present in bridge vectors, signal that human health risk is imminent
	Physician reports	No	Yes	Human cases	Realized human health threat
	Disease history	No	Yes	Number of human/ important animal cases in prior years	Indicates that local conditions are favorable for pathogen amplification and transmission
	Avian dispersal/migration patterns	No	Yes	Time of year regarding dispersal of hatch year birds and known migration periods	Identifies new areas for concern, signals need to control known bridge vectors
Climatic Parameters	Current weather	Yes	Yes	Temp = 65+ Wind < 10 mph No rain	Application time decision
	Short-term weather forecast	Yes	Yes	Presence of fronts & storms; barometric patterns	Application planning
	Time of year	Yes	Yes	Spring, Summer, & Fall activity patterns for trapped mosquitoes	Species-specific behavior; generally, cooler weather retards activity, warmer weather increases activity; virus presence not as significant when activity decreases

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Ecological Parameters	Environmental factors in target area	Yes	No	Environmentally sensitive settings (R-T-E species)	Prior mapping is essential to clearly identify all environmentally sensitive areas; usually addressed through NYSDEC; Town and other expert cooperation is sought
	Population	Yes	Maybe	Number of impacted people/population density	For Vector Control: no people means no problem; for Health Emergency, threat may be sufficient
	Application restrictions	Yes	In some settings	Farms; no-spray list; NYSDEC wetlands, wetlands buffers; open water buffers	Vector Control no-spray areas include crop areas, no-spray list, buffers – discontinuities may make application ineffective

Adulticide applications that are made to reduce vector populations and relieve quality of life impacts will be required to conform to the following four criteria:

1. Evidence of mosquitoes biting residents (there is no problem unless people are affected):
    - Service requests from public - mapped to determine extent of problem
    - Requests from community leaders, elected officials
2. Verification of problem by SCVC (service requests must be confirmed by objective evidence):
    - New Jersey trap counts higher than generally found for area in question (at least 25 females of human-biting species per night).
    - CDC portable light trap counts of 100 or more females of human-biting species.
    - Landing rates of one per minute (measured over a five minute period).
    - Confirmatory crew reports from problem area or adjacent breeding areas.
3. Control is technically and environmentally feasible (pesticides should only be used if there will be a benefit):
    - Weather conditions predicted to be suitable (no rain, winds to be less than 10 mph, temperature to be 65ºF or above).
    - Road network adequate and appropriate for truck applications.
    - "No- treatment" wetlands, wetlands and open water buffers, and no-spray list members will not prevent adequate coverage to ensure treatment efficacy.
    - There are no issues regarding listed or special concern species in the treatment area.

- Meeting label restrictions for selected compounds (such as avoiding farmland) will not compromise expected treatment efficacy.

4. Likely persistence or worsening of problem without intervention (pesticides should not be used if the problem will resolve itself):

    - Considerations regarding the history of the area, such as the identification of a chronic problem area.

    - Determination if the problem will spread beyond the currently affected area absent intervention, based on the life history and habits of the species involved.

    - Crew reports from adjacent breeding areas suggest adults will soon move into populated areas.

    - Life history factors of mosquitoes present – i.e., if a brooded species is involved, determining if the brood is young or is naturally declining.

    - Seasonal and weather factors, in that cool weather generally alleviates immediate problems, but warm weather and/or the onset of peak viral seasons exacerbate concerns.

    - Determining, if the decision is delayed, if later conditions will prevent treatment at that time or not. Conversely, adverse weather conditions might remove most people from harm's way.

In essence, criteria 1 and 2 are necessary thresholds which must be met, prior to a treatment being considered. This means that floor values of 25 mosquitoes per night (New Jersey light traps) or 100 mosquitoes in a CDC light trap **must** be exceeded for vector control to be considered. With enhanced surveillance, there will be rigorous, numeric validation of mosquito control infestation near a potentially affected population in all cases. Treatment will not occur unless criteria 1 and 2 are satisfied through a combination of surveillance indicators, although not all surveillance techniques may be feasible in every setting and situation.

Criteria 3 and 4 are "treatment negation" criteria. If certain conditions are met, treatment will not occur, even if treatment is otherwise be indicated by criteria 1 and 2. Careful records on criteria and thresholds (and related conditions) which trigger each treatment will be kept, for every adulticiding event.

The remaining four parts of the Long-Term Plan discuss how it will be implemented, including:

- establishing an administrative structure for vector control activities in both the Department of Public Works and in the Department of Health Services,
- describing how the technology necessary for implementation should be evaluated,
- developing a framework by which the Long-Term Plan can be adaptively managed (including continuation of the Steering Committee, Technical Advisory Committee, and the Citizens Advisory Committee), and
- estimating the costs to the County of adopting the Long-Term Plan.

One last section specifically describes actions that should be taken to enable implementation of the Long-Term Plan.

Improved Vector Control

An evaluation report on surrounding vector control agencies and Suffolk County's current program described the current program as one of the finest in the Northeast. Nonetheless, there was room for improvement, and the Long-Term Plan proposes to improve essentially all aspects of the current program, from public outreach, to data management, to pesticide applications. As has been mentioned, the adoption of more progressive water management techniques, which the County was by-and-large unable to implement earlier due to resource and regulatory issues, may reap the greatest benefits. However, all aspects of the Long-Term Plan will combine to reduce the risks to County residents from disease and mosquito control activities, and also lead to environmental improvements that may be an invaluable legacy to future generations of County residents.

Interagency Cooperation and Public Input

This Long-Term Plan was the product of an open and extraordinary process that sought, and received, input from local, State, and Federal government agencies and officials, from non-governmental organizations concerned about mosquitoes, pesticides, and wetlands, and from concerned individual citizens and citizen groups. Suffolk County has invested a great deal of money and time into the Long-Tem Plan, but could not have reached these goals without the input, suggestions, and hard work from many others, which it gratefully acknowledges. Overview Table 2 lists major accomplishments of the Long-Term Plan to date, and outlines how some of the technical elements led to components of the plan.

Overview Table 2. Long-Term Plan Accomplishments to date (October 2006)

Plan Element	**Accomplishment**	**Discussion**
Public Education & Outreach	Project website Literature Search National conferences Associated committees	All project output: www.suffolkmosquitocontrolplan.org 1300 pages, with extensive expert review SETAC, AMCA, LI Geologists TAC, CAC, Wetlands Subcommittee
Surveillance	Trap network improvements Enhance larvicide & adulticide efficacy monitoring WNV monitoring re-evaluation EEE conceptual reevaluation	Refinement of a program widely acknowledged to be excellent. The result of literature search and input from national experts.
Source Reduction	Stormwater management expansion from 15,000 to ~50,000 sites	Result of Early Action project
Water Management	Wertheim OMWM Seatuck and Wertheim retrospective studies Salt marsh mapping Identification of unditched marshes Salt marsh extent Wetlands Subcommittee BMP manual Wetlands Management Plan Conceptual re-evaluation of marsh systems	Designed, permitted, began construction on 80 acre salt marsh restoration Early Action project: long-term influences on salt marsh health First GIS map of Suffolk marshes to name them all Recalculated County salt marshes (17,000 acres) Collaboration between Towns, County, NGOs on wetlands Design manual for Suffolk County OMWMs, including tie-ins between mosquito control, wetlands restoration, and Phragmites control Plan to achieve salt marsh management Recognition of uniqueness of each marsh system
Biocontrols & Other Alternatives	Field tests (garlic & rosemary oils, Mosquito Magnet)	Barrier systems & mosquito trap evaluations
Larvicides	Caged Fish experiment Benthic survey Paired marsh invertebrate experiment Risk assessment of 3 current products	Field test of larvicide impacts; included fate & transport Statistical comparison of treated and untreated invertebrate populations 5 pairs of marshes compared for invertebrate impacts Calculation of human health and ecological impacts
Adulticides	Minimize usage, optimize control: Application methodology re-evaluation Caged Fish experiment Adapco Wingman system Risk assessment on current and potential products	 Modeling revealed means to reduce off-target drift Field test of adulticide impacts; included fate & transport Purchased computer model to optimize pesticide applications Calculation of human health and ecological impacts
Project Management	GIS construction Data management re-evaluation Public outreach emphasis Personnel and capital needs evaluation Remote sensing evaluation	Digitized and mapped SCVC records in relational databases First digitized tidal wetland map Digitized 21 PSAs characteristics Need to communicate program effectiveness better ABDL BSL-3 recommendation, staff augmentation, marsh restoration equipment identification Can provide cost-effective coastal marsh monitoring

Three major elements of the project have been three major field programs (the Wertheim OMWM, the Caged Fish experiment, and the characterization of 21 Primary Study Areas in fresh and salt wetlands), the literature search, and the quantitative risk assessment. The literature search resulted in the completion of 26 separate reports, concentrating on the topics of Suffolk County mosquitoes, mosquito-borne disease, vector control, pesticides, and salt marshes. The literature search directly led to major operational improvements such as purchase of the Adapco real-time modeling and weather monitoring system for adulticide application guidance. This state-of-the-art system optimizes mosquito control while minimizing pesticide usage. The literature review also demonstrated that progressive water management, as practiced throughout much of the northeast US, can be successful as a mosquito control technique, and also used as a restoration means for salt marshes. The Literature Search proved to be the technical heart of the project.

In addition to the Literature Search, other written reports were produced as products of other specific project tasks. By far the most important of these was the quantitative risk assessment. This document related the quantifiable risks of impacts to human health and the environment from three currently used larvicides and four adulticide products, based on extensive modeling of pesticide applications, the subsequent fate of those products, and the receptors (human and ecological) that could be affected by their use. Associated with this quantitative assessment were evaluations of impacts from mosquito-borne disease, proposed water management actions, and impacts to human health and the environment from other pesticides use.

Ultimately, the risk assessment demonstrated that each year, in the absence of vector control, it is possible that 16 people might die in Suffolk County from WNV, and another 150 or so might become seriously ill. Risks from EEE, while not quantified, included opportunity for the most efficient mosquito vector of this disease to become infected, and so potentially result in human illness where one-third to three-quarters of those people who become infected might die. Conversely, the risk assessment of pesticides found little to no risk for adverse human health impacts because of the relatively low exposure experienced by people to these control agents. Of the control agents evaluated, only the adulticides posed potential ecological risks. One possible impact is to flying non-target insects, using extremely conservative worst-case assumptions. No actual impacts have been documented in literature, and a California study showed no impacts

and suggests that mitigation is feasible. None of the agents evaluated showed any aquatic ecosystem impacts, with the possible exceptions of permethrin and malathion (which are not expected to be front-line agents, as the County anticipates implementing the Long-Term Plan).

The Wertheim OMWM project was addressed as a collaborative effort among USFWS, Ducks Unlimited, and the County and its consultants. Two years of negotiation and discussion with NYSDEC resulted in a permit application that the State, the County, and USFWS were all satisfied with. Approximately half of the reconstruction was accomplished in March 2005; the remainder has been accomplished in the first few months of 2006. Extensive, comprehensive, and long-term monitoring efforts, begun two years before construction and to be continued in accordance with the permit issued by New York State Department of Environmental Conservation, carefully document this project. The County is hopeful that this project's initial success and the results of the ongoing comprehensive monitoring effort will be persuasive, in light of the documented achievements of similar projects elsewhere in the northeast US, to allow implementation of the aggressive water management program outlined in the Wetlands Management Plan and Section 4.3 of the Long-Term Plan.

The Caged Fish experiment was intended to be a field exercise to document effects of pesticides on representative fish and invertebrates. It evolved into a major effort to document the fate of applied pesticides from release to degradation, and to understand impacts to sentinel organisms in the water column. The effort required modeling, air sampling, efficacy tests, fish and shrimp survival and non-lethal impact studies in the field and laboratory, testing of approximately 100 samples of pesticides in water, sediments, and biota, to the parts per quadrillion level in some cases, using highly specialized equipment and research-level techniques, and multivariate statistical tests to determine the import of all the data. The effort was led by researchers from Stony Brook University, Southampton College, USGS, and the County Public and Environmental Health Laboratory, together with other County employees and the Long-Term Plan consultant team.

In addition, the collaborative nature of the management plan process, with participation by interested parties in local, state, and federal government, and non-governmental organizations with both local and national ties, created a dynamic planning process that allowed for concerns to

be raised, suggestions to be made, and expertise to be interjected. The Long-Term Plan is thus more than a critical examination of past County practices. It is the fruit of a complete reconsideration of vector control intentions and capabilities, in light of practical constraints based on available technology and existing infrastructures. The Long-Term Plan offered here is intended to implement a state-of-the-art example of Integrated Pest Management, where the twin goals of reductions in risk to human health and increases in environmental quality can both be achieved.

Note:

This Revised Long-Term Plan is an update of the Long-Term Plan dated May 3, 2006. Revisions were made to the Long-Term Plan in light of the many and well-considered comments offered to the County through the SEQRA process, primarily as comments on the Draft Generic Environmental Impact Statement (May 3, 2006) for the Long-Term Plan. In this revision, numerous changes were made to the text of the Long-Term Plan, including correcting misspellings or statements of fact. The more substantial changes of substance and tone included the following:

Section 1: p. 36: identification of changes made in the water management approach

p. 38: Goal 2, Objective 1 was modified to reflect the need for an Integrated marsh management program

p. 39: Goal 2, Objective 3 was modified to reflect changes made in the primary wetlands management project review organizations

Section 2: pp. 60, 62: educational program changes

Section 3: pp. 78-79: refinement of landing rate procedures

Section 4: pp. 104-118: substantial changes made regarding wetlands management project identification, review, implementation, and evaluation

Section 7: Table 18, pp. 190-192: consistency in mosquito counts as triggers for action

Inclusion of Figure 7 (mistakenly not included in the original text)

Section 10: pp. 250-255: proposed content of the Triennial Report

p. 256: identification of additional SCVC-ABDL reporting requirements

pp. 257-258: discussion of how the Integrated Marsh Management program may change the Wetlands Management Plan

Section 11: p. 259: identification of the need to provide staff support to the Wetlands Stewardship Committee

Section 12: the above changes are reflected in this section

1. Goals and Objectives

1.1 Goals of the Long-Term Plan

The Suffolk County Vector Control and Wetlands Management Long-Term Plan has two goals:

1. decrease risks to human health and impacts to public welfare from mosquitoes and mosquito management

2. simultaneously reduce impacts to the environment and increase potential ecological benefits associated with the selected management techniques

These goals will be achieved by adopting a progressive mosquito management approach based on the principles of Integrated Pest Management (IPM). The Long-Term Plan does not propose to attempt the futile task of eliminating all mosquitoes from Suffolk County. Not only is that not possible, but it is not desirable, either. Rather, the Long-Term Plan is a roadmap that will allow Suffolk County to address mosquito problems – instances where mosquitoes threaten public welfare and human health. Under such conditions, where control is deemed to be required, the Long-Term Plan uses a hierarchical approach to mosquito management:

- scientific surveillance to determine the locations and types of mosquito problems

- source reduction, including the use of water management to modify habitat to minimize mosquito breeding, is paramount

- when breeding occurs, larval control using products that have no human health effects and little environmental impacts will be undertaken

- if mosquitoes develop into adults, and an assessment of the problem finds that adult control is required, then products will be used that have little to no impact to people, have an acceptably small impact to non-target organisms, degrade quickly, and are effective at killing adult mosquitoes

1.2 Discussion

Suffolk County currently follows the IPM hierarchical approach in its mosquito control program. The County intends, through adoption of the Long-Term Plan, to reduce risks to its residents and improve overall County environmental quality through improvements in each of the major elements of IPM.

It is essential that professional, scientific surveillance of potential mosquito problems be undertaken. Without timely information of the highest quality, it is difficult to reach optimal decisions concerning mosquito control, and to generate public confidence in the decisions so made. Surveillance activities are intended to:

- describe the species and numbers of mosquitoes present in areas of concern
- accurately define the locus of mosquito activity
- document the stage of the mosquito, if immature, or its parity (whether or not it has laid eggs before), if adult
- determine the presence of pathogens in host and sentinel species and mosquito vectors (including amplification and bridge vectors, if relevant) (amplification vectors serve to increase the prevalence of disease in hosts, and bridge vectors transmit disease from hosts to humans)

Data collected in the field will be processed to information quickly, and, if possible, locally. It will then be disseminated to the proper officials in a format that will enable the information to guide control decisions regarding identified mosquito problems.

A mosquito problem is caused by a threat of disease and impacts to public welfare. Worldwide, mosquitoes are identified as the most important vector of human disease. Most of the human misery and death caused by mosquitoes is from the transmission of malaria. Fortunately, Suffolk County and the rest of the US managed to control and nearly eliminate this disease more than half a century ago. Although minor outbreaks of the disease still occur, the risks of malaria to

Americans today are almost non-existent. Similarly, other dread mosquito-borne diseases such as dengue fever and yellow fever are of only passing concern.

The mosquito-borne diseases of concern in Suffolk County right now are encephalitic arboviruses. The two of most concern are Eastern equine encephalitis (EEE) and West Nile virus (WNV). Outbreaks of EEE, which can have fatality rates ranging from 35 to 75 percent, have occurred recently in New Jersey, and in Massachusetts and New Hampshire. Although there has never been a diagnosed human case of EEE in Suffolk County, horses have died from the disease here as recently as 2003, and nearby in Nassau County in 2005. In 1999, WNV was introduced into the country, with the first human cases and deaths occurring in Douglaston, Queens. WNV has now been found throughout the continental US, resulting in over 16,000 human cases with 665 deaths through 2004; four of the people who died were residents of Suffolk County. These encephalitides not only have the potential to kill otherwise healthy individuals, but non-fatal impacts can include neuro-invasive effects, which can be permanent.

It is also clear that there are numerous other mosquito-borne diseases that currently are not found in the US. The immediate lesson of WNV in Suffolk County is that local mosquitoes have the capacity to transmit exotic pathogens, and therefore these diseases pose a significant although unrealized health threat. It is understood that the introduction of a novel mosquito-borne disease here is not a question of "if," but rather a question of "when." This is because modern transportation has removed geographical isolation as a protection from exotic agents. Along with generating undeniable benefits, this facet of modern life also means that disease organisms are often only one airplane flight away.

In temperate climates, human disease is the end-product of a long series of epidemiological events that build in intensity over a period of months. The development of human illness due to this progression can be aborted by careful actions taken to control the disease vectors. Almost all public health plans recognize that waiting for disease to become evident in people means that control efforts begun at that time may be ineffective in preventing further human suffering. This is especially true for mosquito-borne diseases. Mosquitoes tend to be concentrated as immature organisms and targeted control of larvae using natural predators or narrow-spectrum agents are very effective. As adults, mosquitoes tend to widely disperse, complicating efforts to alleviate

the threat of harm, and often requiring the use of chemicals that may have wider non-target impacts.

Therefore, disease control efforts cannot begin when pathogens are circulating in adult mosquitoes. An integrated control program is required for efficient and proper control of endemic diseases such as WNV. Comprehensive surveillance can document areas that pose the greatest risk of disease amplification and transmission. Source reduction should be employed to reduce breeding opportunities for the amplification vectors (if possible) and for those bridge vectors that may eventually pose a risk to human populations. Similarly, larval control needs to be conducted prior to detection of the virus in adult mosquito populations, as larval population reduction efforts will not decrease the imminent risk posed by pathogen presence in amplification or bridge vectors. An integrated program such as this acknowledges that any need for adulticide applications signals failures in other, better means of disease suppression. Thus, because WNV will likely occur in multiple sites in the County every year, with its ultimate geographic distribution apparently the result of complicated interplay and feedback between weather and mosquito, avian, and viral population dynamics, mosquito control conducted for the purpose of preventing cases of human disease needs to be conducted generally across the County and throughout the season.

Nonetheless, Federal and State guidelines have established separate protocols for addressing increasing risks from WNV and other mosquito-borne diseases. These include guidance on how to increase vigilance prior to the introduction of the disease to the general area, and also discuss ways to consider managing increasing risk in a season when the pathogen is detected locally. As part of the process, when imminent risk reaches a certain level, the County Commissioner of the Department of Health Services is authorized to petition for a State Department of Health declaration of a "health emergency." This declaration changes certain lines of local authority (making mosquito control explicitly the responsibility of the Department of Health Services) and allows certain State permitting procedures to be expedited. But the declaration does not signal the initiation of local interest in mosquito-borne disease, nor the beginning of control efforts focused on pathogen transmission. These activities must be an essential part of County vector control activities throughout the year.

Mosquitoes impact public welfare not only by disease transmission, but also through subclinical effects of mosquito biting. Mosquitoes are known to be infected by other viruses, bacteria, and pathogens and parasites, such as worms of various kinds, some of which are implicated in human illness. The salivary fluids released when a mosquito bites typically cause welts, and can cause rashes and various allergic reactions. Thus, even in the absence of defined diseases circulating in mosquito populations, human-biting mosquitoes can adversely impact public health.

Surveillance programs, especially post-WNV introduction, are designed to detect early signs of pathogens, and to determine if health risks presented by disease require actions to reduce the chance of human illnesses. However, human-biting mosquitoes come into contact with blood when they bite. In areas where there is disease transmission risk, the distinction between mosquito control for public health protection and mosquito control for the relief of human discomfort (sometimes called nuisance control) becomes unclear. Nearly all human-biting mosquitoes in Suffolk County have some vector capability for the arboviruses that are the modern day health threats in the northeast US. Thus, control of these human-biting mosquitoes is undertaken to have some impact on the overall risk of disease. Actions taken to reduce the populations of human-biting mosquitoes in Suffolk County reduce the risk of disease transmission, and result in public health benefits beyond minimization of subclinical effects. In addition, there is an ancillary, but important, improvement in the quality of life for those who live, work, or recreate where these mosquitoes live. For parts of Suffolk County, especially in areas in close proximity to the south shore, high numbers of mosquitoes that are very persistent and fierce in their search for blood meals (these are largely spawned from local salt marshes) can make it impossible to spend any amount of time outside, in the absence of mosquito control programs.

Public health protection emphasizes monitoring for pathogens among amplification vector populations, and controlling important bridge vector populations through source reduction (especially water management for salt marsh species), larval control where source reduction is not possible or was not effective, and, if a health risk assessment deems it necessary, adult control. There is significant overlap between this approach and the alleviation of severe public welfare effects. Historically, Suffolk County significantly reduced mosquito populations, particularly along the south shore, through its ditch maintenance program augmented by regular

use of larvicides. The Long-Term Plan proposes to pursue more progressive marsh restoration management practices, which should reduce the need for larvicide applications immensely.

State and County Public Health Law (PHL) identify mosquito control and the reduction of mosquito habitat (such as standing water) as abatement of public health nuisance. A public health nuisance is, by definition, a condition that adversely affects public health (irrespective of whether it causes fatal disease or some sublethal impacts). In this case it is the recognition of health effects from an ectoparasite (mosquitoes are grouped as such with pests such as lice, fleas, and bedbugs). Under State law, health officers have a duty to address the effects caused by these to the public. The presence of pathogens in mosquitoes is not required for this definition of public health nuisance, as the law implicitly recognizes there are health concerns that extend beyond the transmission of diseases such as WNV and EEE.

The Long-Term Plan uses the term "vector control" to describe adulticide applications in the absence of a detected pathogen. In general, "vector control" is interchangeable with "public health nuisance control," as these instances of adult control take place under conditions where there is a low imminent public health threat of the outbreak of serious disease (such as WNV or EEE), where the risk to the public cannot be said to be zero, and where sublethal impacts also occur.

The mosquitoes of Suffolk County develop in both fresh and salt water environments. In order for pathogens of present-day concern to become prevalent enough to pose a major health threat, they need to be amplified through avian reservoirs by fresh water mosquito species. The County, therefore, as it is allowed under regulations that protect important fresh water natural resources, conducts surveillance and control programs to reduce overall health risks. For EEE, it is clear that other mosquito species are needed to spread disease to people, and some of the most able of these species breed in salt water settings. For WNV, the cycling of the pathogen is less well understood, but quite a few fresh and salt water mosquitoes have been determined to be (or are suspected of being) human vectors. Therefore, the integrated control program that focuses on reducing these human-biting mosquito populations, in both fresh and salt water environments, reduces overall risks of disease transmission.

Modern vector control efforts also have a focus on reducing impacts associated with controlling mosquitoes. The County will seek to implement progressive means of water management that will enable it to significantly reduce the pesticide application events as well as the overall amounts of and areas affected by larvicides. Extensive source reduction efforts will also be conducted to reduce upland breeding of amplification and bridge vectors. These efforts are anticipated to reduce the places, application events, and overall amounts of and areas affected by adulticide used in the County. Decisions to use adulticide are not necessarily determined only by the number of mosquitoes. Adulticides are often used to reduce any explicit health threat the mosquitoes may represent.

The pesticides considered for use by the County today for mosquito control have been shown to have little to no risk of health effect on humans, even when exposures are projected for entire lifetimes. At the concentrations that they are applied at, according to most scientific investigations, the formulations have no apparent acute effects on humans, chronic effects are generally found to be of little concern, and the risk of cancer has been estimated to be much less than the level of regulatory concern for the US Environmental Protection Agency (USEPA). This is largely because these chemicals are designed to affect insects, and are especially targeted at mosquitoes. Thus, their modes of action tend not to have any effect on human systems. Most are applied at low concentrations due to the relative fragility of the mosquito (compared to other, agricultural pests that typically require much higher concentrations to be controlled). These pesticides are made to degrade quickly in the environment, so that the amount any person is exposed to, and the time period that an application can affect people, are both extremely small. These same traits limit impacts to non-target organisms present in the environment. Modeling shows that there is a small risk from some adulticides to specific organisms, such as insects flying when the application occurs, and certain invertebrates in exposed aquatic habitats. However, modeling of ecological impact and local monitoring for effects have found no measurable long-term impacts to the ecosystem. In addition, measurements of actual pesticide concentrations following applications in Suffolk County show that modeling may use unduly conservative assumptions regarding the pesticides' environmental persistence. Therefore, there may actually be no measurable impacts to the environment associated with the use of most modern mosquito control pesticides and little increase in risks to the local ecology when they are applied.

Progressive water management in salt marshes achieves its ends by improving habitat for fish and allowing them access to areas where mosquito larvae hatch, or by eliminating isolated breeding areas. This generally results in better water circulation and connection with the nearby estuary, and increases in open water habitats in the high marsh area. Ancillary benefits can include reductions in invasive *Phragmites* and greater use of the marsh by water fowl and piscivorous birds, and predatory fish and crustaceans. Better water quality can sometimes be achieved through removing man-made restrictions to tidal flows. In these ways, progressive vector control practices can be elements of restoration actions for degraded or threatened wetlands, and so produce overall environmental improvement. Careful, scientific selection of appropriate water management techniques in light of overall County management aims will result in healthier marshes County-wide, resulting in greater ecological diversity and productivity for our precious salt marshes and the estuarine systems associated with them. Suffolk County is embedded in the marine environment, and the waters that surround us are cherished and important to us all. Improvements to water management procedures for mosquito control will lead to measurable enhancements of these natural resources.

Many concerns were voiced regarding the proposed water management program, despite how its elements have all been successfully applied in other nearby jurisdictions over the past forty years. Respecting the deep passion for the environment that sparked these comments on the original County approach, the County has enhanced the review process associated with its water management program. Water management for the purposes of mosquito control will be put in the context of a comprehensive marsh management program and Integrated Marsh Management program that will incorporate all aspects of coastal marsh management. In addition, the County has slowed its timetable for implementing marsh management considerably. The County still anticipates conducting marsh management for mosquito control purposes, however, because successful implementation of these techniques promise that there will not only be less pesticide use in these marshes, but also improved ecological functioning of the restored areas.

1.3 Objectives

Explicating a set of well-defined objectives for the Long-Term Plan provides the means for the interested public to understand how the ambitious Goals of the Long-Term Plan will be achieved. The following are the objectives for the two goals.

Goal 1: Decrease risks to human health and impacts to public welfare from mosquitoes and mosquito management

Objective 1. The prevention of serious disease in residents of and visitors to the County, as practical, is of utmost importance.

Objective 2. Problem populations of mosquitoes will be reduced where possible (when exceeding threshold/criteria described in Section 7, below) because large numbers of human-biting mosquitoes, in association with people and areas where mosquito-borne diseases have been detected, represent increases in overall health risks for those people. Enhancement of public welfare is an important auxiliary benefit. This objective relates to "Vector Control:" in the chance of a detected pathogen, this can also be considered "Public Health Nuisance Control."

Objective 3. To achieve these objectives, the County's program will follow the principles of IPM, seeking to address mosquito problems by means of appropriate controls applied at times of greatest effectiveness and least impact to human health and the environment.

Objective 4. A program of scientific surveillance will be employed, with the intent of accurately and specifically defining potential mosquito problems.

Objective 5. Source reduction will be the primary focus of mosquito control. A key element will be public education, outreach, and assistance for habitat reduction around homes and businesses. The second key element is the adoption of a program of Best Management Practices and, in appropriate areas, progressive and extensive water management projects, to be implemented in coordination with (and with approval from) local and State agencies, and with the participation and oversight of other stakeholders.

Objective 6. The use of biorational larvicides, specifically targeted towards larval mosquitoes, will allow for reduction of any identified mosquito problem prior to dispersal as adults, when control is more difficult.

Objective 7. The use of adulticides, when all other methods of control have been unsuccessful or when other control methods cannot be implemented, if Vector Control (Public Health Nuisance) thresholds are exceeded, or if emergency response conditions exist.

Objective 8. The mosquito control program will be guided by an appreciation for the overall management of risk to people, minimizing potential impacts to human health from disease and from control methods.

Other ancillary benefits of the Long-Term Plan are to facilitate enjoyment of the County's natural environments, and to support local businesses and enterprises that depend on tourism and recreation, as is possible while also attaining the specified objectives of the Plan.

Goal 2: Simultaneously reduce impacts to the environment and increase potential ecological benefits associated with the selected management techniques.

Objective 1. The County will adopt an overall plan for Integrated Marsh Management based on a definition of marsh health that will emphasize the need to preserve or increase acreage of wetlands, including vegetated wetlands, and to foster biodiversity and a mosaic of ecological communities. Vector control efforts will be accommodated within this framework, but will not necessarily be the primary determinant in marsh management decision-making. In salt marshes, most areas will either be subject to reversion or low impact Best Management Practices. In certain areas, the judicious employment of progressive water management will be continued, with the intent to increase overall habitat diversity, generated by an ecological setting composed of tidal creeks, ponds, low and high marsh, pannes, mudflats, salt shrub, associated freshwater wetlands, and adjacent beaches or sand berms. This will provide a variety of microhabitats and ecotones, which should support appropriate plant and animal diversity, as measured by monitoring and project evaluations. Projects conducted under the Long-Term Plan will also seek to reduce invasive species, especially *Phragmites*, in the managed wetlands.

Objective 2. The aim of the water management program is to reduce the routine use of larvicides, ultimately resulting in significant reductions in the overall acreage where larvicides are applied each year. Each marsh will be examined on a case-by-case basis, and major decisions of marsh management projects must be reviewed and approved by the Wetlands Stewardship Committee. Biodiversity, vector control, and *Phragmites* control are all important marsh management goals. Each needs to be considered for all projects. For example, marsh restoration projects may be implemented for biodiversity purposes, with design elements that achieve net mosquito-neutral effects. Other projects will be considered because they will reduce mosquito populations and potentially create environmental benefits. The initial list of priority salt marshes for consideration for progressive water management, however, is comprised of those sites where aerial applications of larvicides are currently used to treat mosquito breeding.

Objective 3. To ensure that water management projects achieve natural resource goals, the County intends to continue to rely on review of projects by local natural resource personnel, the Wetlands Stewardship Committee, and regulators such as New York State Department of Environmental Conservation (NYSDEC).

Objective 4. Where mosquito breeding occurs despite water management efforts, or where no such actions can be taken, biorational larvicides will be used to ensure that no or, at worst, minimal, non-target impacts to the surrounding ecosystems.

Objective 5. If adult mosquito population control proves to be necessary, the County will use adulticide products that have no significant, long-term impacts to the environment.

Objective 6. The mosquito control program in general will be guided by an appreciation for the overall management of risk, minimizing potential impacts to the environment and natural systems and improving them where possible, while protecting human health and public welfare.

1.4 Mosquitoes of Suffolk County

Table 1 lists the 50 mosquito species found in Suffolk County. This list has been compiled through trapping and literature analyses by the Suffolk County Department of Health Services (SCDHS) Arthropod-Borne Disease Laboratory (ABDL), Dr. Scott Campbell, Director.

Table 1. Mosquitoes of Suffolk County

	Reinert (2000)	**WRBU (2005)**
1	*Aedes cinereus*	*Aedes cinereus*
2	*Aedes vexans*	*Aedes vexans*
3	*Anopheles barberi*	*Anopheles barberi*
4	*Anopheles bradleyi*	*Anopheles bradleyi*
5	*Anopheles crucians*	*Anopheles crucians*
6	*Anopheles earlei*	*Anopheles earlei*
7	*Anopheles punctipennis*	*Anopheles punctipennis*
8	*Anopheles quadrimaculatus*	*Anopheles quadrimaculatus*
9	*Anopheles walkeri*	*Anopheles walkeri*
10	*Coquillettidia perturbans*	*Coquillettidia perturbans*
11	*Culex erraticus*	*Culex erraticus*
12	*Culex pipiens*	*Culex pipiens*
13	*Culex restuans*	*Culex resturans*
14	*Culex salinarius*	*Culex salinarius*
15	*Culex territans*	*Culex territans*
16	*Culiseta annulata*	*Culiseta annulata*
17	*Culiseta inornata*	*Culiseta inornata*
18	*Culiseta melanura*	*Culiseta melanura*
19	*Culiseta morsitans*	*Culiseta morsitans*
20	*Culiseta silvestri minnesotae*	*Culiseta silvestri minnesotae*
21	*Ochlerotatus abserratus*	*Aedes abserratus*
22	*Ochlerotatus atropalpus*	*Aedes atropalpus*
23	*Ochlerotatus aurifer*	*Aedes aurifer*
24	*Ochlerotatus canadensis*	*Aedes canadensis*
25	*Ochlerotatus cantator*	*Aedes cantator*
26	*Ochlerotatus diantaeus*	*Aedes diantaeus*
27	*Ochlerotatus dorsalis*	*Aedes dorsalis*
28	*Ochlerotatus excrucians*	*Aedes excrucians*
29	*Ochlerotatus fitchii*	*Aedes fitchii*
30	*Ochlerotatus flavescens*	*Aedes flavescens*
31	*Ochlerotatus grossbecki*	*Aedes grossbecki*
32	*Ochlerotatus hendersoni*	*Aedes hendersoni*
33	*Ochlerotatus intrudens*	*Aedes intrudens*
34	*Ochlerotatus japonicus japonicus*	*Aedes japonicus japonicus*
35	*Ochlerotatus sollicitans*	*Aedes sollicitans*
36	*Ochlerotatus sticticus*	*Aedes sticticus*
37	*Ochlerotatus stimulans*	*Aedes stimulans*
38	*Ochlerotatus taeniorhynchus*	*Aedes taeniorhynchus*
39	*Ochlerotatus triseriatus*	*Aedes triseriatus*
40	*Ochlerotatus trivittatus*	*Aedes trivittatus*
41	*Orthopodomyia alba*	*Orthopodomyia alba*
42	*Orthopodomyia signifera*	*Orthopodomyia signifera*
43	*Psorophora ciliate*	*Psorophora ciliata*
44	*Psorophora columbiae*	*Psorophora columbiae*
45	*Psorophora confinnis*	*Psorophora confinnis*
46	*Psorophora ferox*	*Psorophora ferox*
47	*Psorophora howardii*	*Psorophora howardii*
48	*Toxorhynchites rutilus septentrionalis*	*Toxorhynchites rutilus septentrionalis*
49	*Uranotaenia sapphirina*	*Uranotaenia sapphirina*
50	*Wyeomyia smithii*	*Wyeomyia smithii*

Reinert, JF. 2000. New classification of the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera and notes on certain subgenera and species. *Journal of the American Mosquito Control Association* 16:175-188.

WRBU. 2005. *2001 Systematic Catalog of Culicidae*. Walter Reed Biosystematics Unit. www.mosquitocatalog.org/main.asp. Retrieved June, 2005.

Not all of the mosquitoes on the list are of concern for people. Mosquitoes that do not impact people either through biting or disease association are sometimes labeled as scientific curiosities. However, even these mosquitoes can become of interest as conditions change. *Culiseta melanura* was once treated as a curiosity because of its strange overwintering habitat in the roots of trees in swamps. Currently, this mosquito is subjected to intense surveillance, as it plays an essential role in the amplification of EEE.

All mosquitoes require water to breed in. Because mosquito larvae are air-breathing organisms and poor swimmers, they do not tolerate moving water that does not allow them to establish their siphon to the atmosphere, and so quiescent or standing water is where mosquito breeding occurs. Certain mosquitoes emerge as adults at the end of winter, but in Suffolk County, the predominant species of management concern are those that breed and hatch during warm weather. Some mosquitoes tolerate salt water, and so their larvae grow in pooled water on salt marshes (salt marsh mosquitoes). Others are found in natural fresh water environments, and still others have adapted to man-made settings, especially discarded objects that hold water, whether temporarily or permanently.

Shallow still or slow-moving waters best suit mosquito larvae. This means marshes and swamps are favored habitats. Mosquito problems today are much less than they were formerly, primarily because we have destroyed so much of the original extent of wetlands across Suffolk County and the nation in general. Appreciation for these habitats has been generally fostered (and codified into law and regulation) over the past 50 years or so. This means that source reduction as a means of mosquito control is more carefully addressed than it used to be.

Table 2 contains a list of 15 species of concern in the County. The mosquitoes have been classified in terms of their vector capability and/or impacts to quality of life, along with pertinent environmental factors. It is clear that it is difficult to separate the mosquitoes that serve as disease risks from those that are of concern for the spread of disease. This is partially because aggressive biting behavior is a characteristic that is likely to make a mosquito species a bridge

vector, especially since birds constitute the major disease hosts for arboviruses. Mosquitoes that are aggressive also tend to be somewhat indiscriminant in their feeding habits, and so create opportunities, if they are capable of serving as a vector for a disease, of spreading that disease from birds to people due to their feeding habits.

Table 2. Mosquito Species of Concern in Suffolk County

Species	**Vector Status**	**Human Nuisance**
Aedes vexans	Known WNV bridge vector Probable EEE bridge vector	Aggressive, SC's major fresh flood water mosquito
Anopheles punctipennis	Possible WNV bridge vector	Fresh water mosquito; pesky, enters houses
Anopheles quadrimaculatus	Malaria vector	Fresh water mosquito, moderately aggressive
Coquillettidia perturbans	WNV and EEE bridge vector	Aggressive nuisance, breeds in emergent fresh marshes
Culex pipiens	WNV amplification vector Probable WNV bridge vector	Breeds near (containers, catch basins, other standing water) and enters houses
Culex restuans	WNV amplification vector	Fresh water mosquito
Culex salinarius	WNV bridge vector	Irritating biter, breeds in brackish flood water and salt marshes (distribution not well understood in Suffolk County)
Culiseta melanura	EEE amplification vector Probable WNV amplification vector	Red-maple and Atlantic white cedar swamps
Ochlerotatus canadensis	Probable EEE bridge vector Possible WNV bridge vector	Spring fresh water mosquito, extremely long lived, avid human biter
Ochlerotatus cantator	Possible WNV bridge vector	Spring salt water mosquito, moderately aggressive
Ochlerotatus japonicus japonicus	WNV bridge vector	Tree-hole (tire) mosquito, causes local nuisance, moderately aggressive
Ochlerotatus sollicitans	EEE bridge vector Probable WNV bridge vector	SC primary pest species, extremely aggressive, salt water flood mosquito
Ochlerotatus taeniorhynchus	Possible WNV bridge vector	Aggressive salt water flood mosquito
Ochlerotatus triseriatus	Possible WNV vector La Crosse encephalitis vector	Irritating pest, containers-tree holes-tires mosquito
Ochlerotatus trivittatus	Possible WNV vector	Aggressive fresh flood water (recharge basins) mosquito

These key species are discussed in some detail below. Mosquito species can be distinguished by certain life cycle characteristics. They may have one generation a year (univoltine) or more than one (multivoltine). Their eggs may require a period of exposure to the atmosphere (desiccation tolerant) or not be able to withstand exposure to air (desiccation intolerant). Larvae may be able to survive in salt water (salt tolerant) or not. More information is available in Book 1 of the Task Three Literature Search, "Long Island Mosquitoes."

Aedes vexans – The Inland Flood Water Mosquito

Aedes vexans is a medium-sized mosquito. It has white, narrow bands on some segments of the tarsi. The third, fourth, and fifth abdominal segments are dark-scaled, with white basal bands and a V-shaped notch posteriorly. The average life span of an adult is three to six weeks.

Ae. vexans is multivoltine, its eggs are desiccation tolerant, and its larvae will overwinter. Eggs are laid in sites subject to inundation by water. Hatching will occur as the result of a reduction in dissolved oxygen content. It takes six to eight days for larval development. Larval habitats include open, shallow grass filled depressions and woodland pools. It is mainly a fresh water mosquito; however, it will breed in salt marshes. Larvae are usually found between mid-April and October depending upon climatic conditions. Adults are present June to late September. *Ae. vexans* has a flight range of five to ten miles from the breeding site.

This mosquito can cause serious disruptions to human activities. It will feed in shady places during the day, but is mostly active at dusk. Peak activity occurs 30 to 40 minutes after sunset. Females bite readily, but not very viciously. Females take blood from whatever hosts are available, and blood feeding begins on the second day after emergence. *Aedes vexans* is a primary vector of dog heartworm. It has also been shown to transmit EEE, Western equine encephalitis, and St. Louis encephalitis in the laboratory. EEE has been found in field collected specimens. *Ae. vexans* is also an identified bridge vector of WNV.

Anopheles punctipennis - The Over-wintering Mosquito

Anopheles punctipennis is a large-sized mosquito, known to fly great distances. It has elongated palps, equal in length to the proboscis. Another identifying characteristic is the distinct yellow markings located at the top and side of each wing. *An. punctipennis* larvae can be found in fresh water swamps, ditches, ponds, springs, pits, puddles, and artificial containers. Larvae preferentially are found in cool and clear water. The larvae will lie on the surface of the water with their bodies parallel to the surface. The greatest abundance of *An. punctipennis* occurs in early spring and late fall. It is present in the summer, but is much less abundant. Oftentimes, adult, inseminated females will overwinter in buildings, cellars, hollow trees, and other protected shelters.

An. punctipennis are vicious biters, can have large impacts on people's activities, and enter houses readily. Females will usually attack after dusk, but will also bite during daytime in a wooded area or at their resting places. This mosquito rests during the day in dark moist shelters. *An. punctipennis* will feed on mammals and birds. It can be infected with malaria in the laboratory, although it is not considered to be a primary malaria vector. *An. punctipennis* was involved in the malaria epidemics during the late 1800s and early 1900s in northern California. It is a very good carrier of dog heartworm, and is a possible WNV bridge vector.

***Anopheles quadrimaculatus* – The Common Malaria Mosquito**

Anopheles quadrimaculatus is a medium-sized mosquito, dark brown in color. The wings are entirely dark scaled, four mm in length, and have four distinct dark-scaled spots. *An. quadrimaculatus* larvae have widely spaced hairs on the head capsule. Larvae are typically found in sites with abundant rooted aquatic vegetation such as rice fields, irrigation ditches, fresh water marshes, lakes, ponds, and reservoirs. The typical larval period is 12 to 20 days and there can be seven to 10 generations per season. The flight range of *An. quadrimaculatus* is one mile or less.

An. quadrimaculatus is a significant pestiferous species. It feeds during the night and rests during the day. It is active for a short period after dusk and just before dawn. This mosquito is most active in the summer, and adult females will overwinter. *An. quadrimaculatus* primarily feeds on mammals. It is the primary vector for malaria in North America, especially in the eastern US. It has also been found to transmit St. Louis encephalitis, in the laboratory. It is an excellent host for dog heartworm and can transmit Cache Valley virus. Pools of *An. quadrimaculatus* have been found to be WNV positive in the US since 2001.

***Coquillettidia perturbans* – The Salt and Pepper Mosquito**

Coquillettidia perturbans is a large, brown and pale speckled mosquito. It is most often identified by pale bands at the outer third of both the hind femur and the hind tibia. *Cq. perturbans* is univoltine, its eggs are desiccation intolerant, and its larvae are found in fresh water. Larvae will attach themselves to the roots of emergent vegetation, which makes larval surveillance and control difficult. This mosquito can overwinter in various stages of larval

development. Larval habitats include permanent ponds, lakes, and marshes. The larvae possess two large air bladders which allow them to store air while underwater for long periods of time.

Adults emerge during spring and summer. Adult females can be vicious biters and will bite during the day in shady, humid places. However, the majority of feeding takes place at dusk and after midnight. Typical hosts for *Cq. perturbans* include humans and other mammals, and birds. This species is a strong flier and will travel several miles searching for hosts. *Cq. perturbans* has been found to be a bridge vector for EEE, and pools have tested positive for WNV. It is attracted to light traps.

***Culex pipiens* – The Common House Mosquito**

Culex pipiens is identified by the basal bands of its abdominal terga. Females have short palpi and a blunt, rather than pointed, abdomen. *Cx. pipiens* is multivoltine, and its eggs are desiccation tolerant. Larvae are found in polluted (organics-rich but fresh) water in cans, buckets, tires, bird baths, rain gutters, wading pools, storm drains, and catch basins. The presence of *Cx. pipiens* adults is an indicator of polluted water in the very immediate vicinity. It can thus be controlled by searching for and removing its larval habitats. This is the species that causes the most human discomfort in urban and suburban settings.

Adult females will overwinter, blood feed in the spring, and then lay eggs that produce the summer populations. *Cx. pipiens* continues breeding throughout the summer; population numbers gradually decline until the first frost. It prefers to feed on birds, but will bite humans and other mammals, as well. It is a primary vector of St. Louis encephalitis and is generally believed to be the prime WNV vector in the northeast US (including Suffolk County) (although some experts disagree). *Cx. pipiens* is the most widely distributed mosquito in the world and can be found on every continent except Antarctica. Individual mosquitoes, however, do not travel far from breeding sites.

***Culex restuans* - The White Dotted Mosquito**

The abdomen of the *Culex restuans* mosquito has straight, pale-scaled basal bands. It may also be identified by its unbanded hindtarsomeres and proboscis. Larval habitats of *Cx. restuans* include edges of grassy swamps, sphagnum bogs, road side ditches, tires, buckets, catch basins,

and septic seepage. Similarly to *Cx. pipiens*, this mosquito can often be controlled by seeking for and removing its breeding habitat. This species is most abundant in the spring and early summer. It is also present during the late summer and autumn, but is less numerous.

Cx. restuans is a night time biter that feeds almost exclusively on birds. It will invade night time roosts, and bite sleeping birds. *Cx. restuans* has been shown to take multiple bloodmeals during each egg-laying cycle, which enhances its virus transmission capabilities, and may increase its importance as an amplification vector. In addition, sampling that speciates *Culex* light trap collections shows that the relative abundance of *Cx. restuans* far exceeds *Cx. pipiens*, suggesting that it is a significant element in the transmission cycling of WNV. Under certain circumstances, *Cx. restuans* may accept humans as a blood meal host, even to the point where it receives attention as a human pest. In most cases however, *Cx. restuans* is not attracted to humans and the species is not regarded as a significant impactor of people's lives. This species may transmit Western equine encephalitis, and EEE has occasionally been isolated from *Cx. restuans.* This mosquito is an important amplification vector for WNV, due to its prey preferences. *Cx. restuans* are more readily trapped with properly baited gravid traps than with light traps. Its flight range is up to one or two miles.

***Culex salinarius* – The Unbanded Salt Marsh Mosquito**

Culex salinarius is a medium-sized mosquito. It can be distinguished from *Cx. pipiens* by its longer, more slender siphon. *Cx. salinarius* is multivoltine, and its larvae have some salt tolerance. They can be found in grassy pools, ditches, ponds, rain barrels, cattle tracks, and stump holes. Larvae are often found in fresh or brackish water that contains emergent and decaying vegetation. Larval populations tend to increase toward the end of summer and are frequently found in atypical habitats later in the season. However, in Suffolk County and coastal Connecticut these mosquitoes have been found to breed in the upper reaches of the irregularly tidally-flooded salt marsh. Population numbers will peak after flooding, and rotting salt marsh vegetation functions as an oviposition attractant.

Adult populations build gradually from spring through summer and do not cease host seeking activities during the autumn. A late season population peak usually occurs which persists until cold weather brings about hibernation. *Cx. salinarius* will overwinter as inseminated, adult

females; therefore, it exits hibernation in a parous state. *Cx. salinarius* will invade open water in Atlantic white cedar swamps directly above the subterranean crypts that support *Culiseta melanura*. Adults rest during the day, often in outbuildings and other similar shelters. They will host seek two to three hours after sunset. *Cx. salinarius* will readily enter houses and can be pestiferous. It is indiscriminate in host choices, readily biting birds and mammals (including humans). These mosquitoes are efficient vectors of St. Louis Encephalitis and WNV (it was identified as the primary vector for WNV in Connecticut). *Cx. salinarius* has been shown to take multiple bloodmeals during each egg-laying cycle, which enhances its virus transmission capabilities, and may increase its importance as a bridge vector. This species is considered to be a good flier, with a flight range of up to five miles. Its prevalence in Suffolk County may have been underestimated, as 2005 identification efforts aimed at discerning whether a Culex mosquito was actually *Cx. restuans*, *Cx. pipiens*, or *Cx. salinarius* found a much higher proportion of *Cx. salinarius* than was expected.

***Culiseta melanura* – The Black-tailed Mosquito**

Culiseta melanura is a medium sized mosquito. It is easy to recognize in the larval stage by the long air tube and prominent antennae. Under the microscope, the unique bar-like comb scales are diagnostic; no other larva has a comb that is even remotely similar. Adult *Cs. melanura* are often mistaken as *Culex*.

Cs. melanura is multivoltine (three to four generations per year), its eggs are desiccation tolerant, and its larvae are found in fresh water. It is present year round. *Cs. melanura* overwinters as larvae. The larvae can be found in underground crypts in acid water bogs with a pH of 5.0 or lower. They are commonly found in Atlantic white cedar and red maple swamps in holes in the flooded root crypts of these trees.

This species is most common in the spring and summer. *Cs. melanura* prefers to feed on birds and is not attracted to mammals. It is the primary amplification vector for EEE, and may serve as an amplification vector for WNV. Control of this species is difficult for several reasons. Habitat access for larval control is very difficult, and, in addition, the swamps where larvae are found are often of regulatory concern due to co-existing rare-threatened-endangered species. Because of its key role in EEE transmission, adulticide use is often countenanced when EEE is

found to be present. *Cs. melanura* are attracted to light traps. The typical flight range is less than one mile.

***Ochlerotatus canadensis* – The Woodland Pool Mosquito**

Ochlerotatus canadensis adults are mostly dark brown. The wing scales are narrow and dark. The legs of *Oc. canadensis* have white double-banding on the tarsi. Narrow basal pale bands on the abdominal tergites are present. Larvae hatch in late winter and spring. Sometimes, a second brood may hatch in mid- to late fall. Its eggs are desiccation tolerant and larvae develop in temporary or semi-permanent shaded fresh water woodland pools containing fallen leaves, or in pools adjacent to wooded areas. *Oc. canadensis* will overwinter as an egg.

Adults emerge in April, May, and early June. This mosquito can live a long time, until late summer. It is a persistent biter, and will bite humans during the morning and evening hours when disturbed. *Oc. canadensis* are indiscriminate in host selection, biting mammals, birds, amphibians, and reptiles. *Oc. canadensis* does have a preference for mammalian blood, but also an affinity for turtles. This species has been identified as a probable EEE and a possible WNV bridge vector. It has been shown to transmit LaCrosse encephalitis and heartworm. *Oc. canadensis* is typically addressed using larvicides. However, if EEE is a concern, because of habitat overlap with *Cs. melanura*, it is often a target for adulticide control. Its flight range is a quarter mile.

***Ochlerotatus cantator* – The Brown Salt Marsh Mosquito**

The maxillary palpus of a female *Ochlerotatus cantator* is less than half the length of either the antenna or proboscis. The postspiracular setae are present and the prespiracular setae are absent. The scales on the dorsal surface of the radial sector and media are narrow. It is multivoltine, its eggs are desiccation tolerant. Larvae are salt water tolerant. It will brood in the upland edge of a salt marsh in spring. Populations from the spring brood generally peak by mid-May and become mixed with those of *Oc. sollicitans*. Breeding, in lesser numbers, continues later in the season, but the larvae become distributed over a wider range of salt marsh habitat. Larvae can generally be found in both salty and brackish habitats well into the fall. *Oc. cantator* larvae resemble *Ae. canadensis* larvae.

Oc. cantator is a persistent biter and an aggressive human feeder. It mainly feeds in the evening, on mammals and birds, but will also bite during the day if its habitat is disturbed. It causes the most problems for people at dusk. *Oc. cantator* has been determined to most probably not be a vector for EEE, and is not believed to be an effective transmitter of WNV, although it is possibly a bridge vector. Control measures that are effective for *Oc. sollicitans* are effective for *Oc. cantator*. This species is attracted to light, and so is effectively caught in light traps (in fact, in many cases, the numbers of *Oc. cantator* in light traps give an over-estimation of the numbers that are actually biting people). *Oc. cantator* has a substantial range, and has been found 10 to 20 miles from larval habitats.

***Ochlerotatus japonicus japonicus* – The Japanese or Rockpool Mosquito**

Adults are very large and easily identified by the eye. The coloration of this mosquito is black throughout, accented by three white leg bands on the hind legs, a gold lyre symbol on the mesonotum, and thin white bands across the abdomen. Larvae can be found in small-volume containers of relatively clean, clear water. They are most often recovered from artificial containers such as bird baths, buckets, plastic milk jugs, wheelbarrows, animal watering containers, and tires. They have also been collected from tree holes, rock pools, cement catch basins, and standing water in tire ruts. It overwinters as larvae. Larvae are present all year long.

Adults have been found from early April through late November. It is most active during the day, but will readily bite humans at night, too. It feeds on a wide array of mammals and birds. *Oc. japonicus japonicus* is an efficient vector of WNV; some believe its importance as a WNV vector is underestimated in the US. It is not known to be an EEE vector, although laboratory testing shows it is capable of doing so. *Oc. japonicus japonicus* is an introduced and invasive species from Asia. This species is now widespread in Suffolk County. It shares many lifestyle characteristics with *Oc. triseriatus,* and is attracted to carbon dioxide (so that CDC light traps are effective means of surveillance). Its flight range is limited to about a tenth of a mile.

***Ochlerotatus sollicitans* – The Eastern Salt Marsh Mosquito**

Ochlerotatus sollicitans is a medium sized mosquito. It is usually identified by a pale-scaled band near the middle of its proboscis, a pale-scaled band on the middle of its first hindtarsomere,

wide tarsal bands, and a pointed abdomen. This mosquito is similar in appearance to *Culex tarsalis*. *Oc. sollicitans* is multivoltine, its eggs are desiccation tolerant, and its larvae are salt water tolerant. Larvae are mostly found in salt marshes, but can also be found in brackish swamps. It overwinters as an egg. Larvae and adults can be found April through October. This mosquito can occur in huge numbers. One production estimate is that each lunar tide (neap or full moon) in summer can result in one million mosquitoes per acre, as each female lays between 100 and 200 eggs.

Oc. sollicitans is a persistent biter and will attack at any time, day or night. It rests in vegetation during the daytime, and will attack something invading its resting areas, even in sunlight. It is the primary cause of human discomfort in Suffolk County, especially on the south shore where large broods can sometimes make normal activities impossible to pursue. *Oc. sollicitans* feeds preferentially on humans and large animals, but also on small mammals and, sometimes, birds. It has been found to be a vector of EEE and heartworm, and is most probably a WNV vector. Coastal mosquito control agencies are often tasked with ensuring this mosquito does not prohibit an outdoors lifestyle for residents near the shore communities, and to minimize its role as a bridge vector. *Oc. sollicitans* are strong fliers; typical published flight ranges are five to 10 miles, although some mosquitoes have been found up to 40 miles from larval habitats. It is strongly attracted to lights.

***Ochlerotatus taeniorhynchus* - The Black Salt Marsh Mosquito**

Ochlerotatus taeniorhynchus is a medium to small sized mosquito. It is identified by white-tipped palps, white ring at middle of proboscis, white basal bands on abdomen and legs, and dark wing scales, and does not have a band at the middle of the first tarsal segment of the legs.

Oc. taeniorhynchus is multivoltine, and its eggs are desiccation tolerant. Larvae develop mostly in salt marshes, but also in fresh water pools. Larvae are also found in inland brackish-water swamps and pools; a particular habitat is in oil fields. Adults emerge six days after the eggs hatch. This mosquito is most abundant during summer and early fall following high tides and/or heavy rains. Eggs enter diapause in response to decreasing day length and water temperature in order to overwinter.

This species is a persistent biter and will attack birds and mammals day and night. It usually rests in vegetation during the day, unless disturbed. While capable of transmitting EEE and St. Louis encephalitis in the laboratory, *Ochlerotatus taeniorhynchus* is not believed to be a major vector of these diseases in nature. It is recognized as an important vector of dog heartworm and Venezuelan equine encephalitis, and appears to be capable of WNV transmission. It is a strong flier, and often migrates in large numbers. Its flight range is five to ten miles.

***Ochlerotatus triseriatus* – The Eastern Tree Hole Mosquito**

Ochlerotatus triseriatus is a medium sized mosquito. It is identified by pale-scaled stripes on the sides of the scutum, unbanded hind tarsi, few hairs on the scutum, and distinct bands on the abdomen.

It is multivoltine, and larvae are found in tree holes and artificial containers, mainly in shaded or wooded areas. Adults reach very high numbers (as many as 60,000 females per acre in mid-summer) in tire scrap yards. This mosquito has become more common in urban areas because it breeds so readily in discarded tires. *Oc. triseriatus* overwinters as an egg. As the weather cools, eggs switch into diapause and will not hatch even if flooded. Larvae are found May through September.

Oc. triseriatus flies and bites during the day in shaded or wooded areas. It is a persistent biter and bites a wide variety of mammals, including humans. Sometimes it feeds on birds, but its preference is for squirrels and chipmunks. This mosquito is the primary vector of La Crosse encephalitis, and is identified as a possible WNV vector. *Oc. triseriatus* is widely distributed throughout North America. Individual mosquitoes have a flight range of less than a mile.

***Ochlerotatus trivittatus* - No common name**

Ochlerotatus trivittatus is a medium sized mosquito. The scutum of this mosquito has a pair of submedian, pale-scaled stripes, separated by a dark strip in the middle. The abdomen has a distinctive triangular pattern. It is also characterized by: unbanded legs, unbanded hindtarsi, a dark unbanded surface on the dorsum of the abdomen, and clear, unspotted wings. *Oc. trivittatus* also has two stripes of white scales separated by a narrow band of dark scales running down the top of the mesonotum.

Oc. trivittatus is multivoltine, and its eggs are desiccation tolerant. Larvae are found in fresh water habitats like flooded woodlands, marshes, open pools, and woodland pools. They are especially common in recharge basins that retain water intermittently. Adults are present from June to September. It is a persistent and aggressive biter, and will bite in bright sun or open areas when its territory is invaded. However, it is most active in the evening. *Oc. trivittatus* prefers to feed on mammals (including humans). It is thought to be a potential vector of WNV, but not to be an EEE vector. Because its flight range is not great (a half mile or less), control efforts often focus on identification of and then elimination or treatment of larval habitat, especially in recharge basins.

1.5 Environmental Settings of Concern

Mosquitoes are aquatic through their larval stages. All mosquitoes need water in order to survive. The additional requirements of their larval life-style mean that the salt marshes and fresh water wetlands of Suffolk County are of special concern as potential environments for mosquito breeding. Cashin Associates quantified 16,839 acres of vegetated salt marsh within the County through a GIS mapping interpretation. The New York State Department of Environmental Conservation (NYSDEC) has mapped 18,084 acres of fresh water wetlands. SCVC has established over 2,000 "breeding points" to monitor on a regular basis for potential control of mosquitoes. However, there are an estimated 100,000 storm water structures along roads in the County, and innumerable half-filled cans, wading pools, poorly-maintained gutters, and abandoned swimming pools, plus thousands of discarded tires, in backyards and throughout the woods, all of which can also serve as sites to breed mosquitoes. Sites as small as a deer hoofprint or as large as 500 acres of salt marsh can serve as focus points for a breeding problem, which makes for a daunting scope of work.

1.6 Legal Authority for Mosquito Management

New York State PHL authorizes agencies to investigate and ascertain the existence and causes of disease outbreaks, including vectors, and to take measures necessary to protect the public health. The New York State Department of Health (NYSDOH) enforces compliance with the PHL. The powers and duties of NYSDOH are set forth in Article 2, § 201 of the PHL. Among these are the supervision of local boards of health and health officers, (PHL § 201[a]), supervision of the

reporting and control of disease (PHL § 201[c]), controlling the pollution of waters of the state (PHL § 201[l]), controlling and supervising the abatement of nuisances likely to affect public health (PHL § 201[n]), and advising any local unit of government in the performance of their duties and regulate financial assistance granted by the state in connection with public health activities (PHL § 201[o]).

PHL Article 15, sections 1520 et seq., authorizes a county to form a Mosquito Control Commission (MCC), and sets forth the powers and duties of said commission. The commission may use appropriate means to suppress mosquitoes, with the limitation that said measures "shall not be injurious to wildlife" (PHL sec. 1525[2]). In Suffolk County, mosquito control was a function of the Suffolk County MCC. That Commission is still referenced in the Suffolk County Charter (SCC), but is no longer active. Amendments to the County Charter in 1973 established the SCDHS. These amendments continued the existence of the Suffolk County Health District, noting therein that the Commissioner of the Department would be the chief administrative officer of the District, and that any reference of the New York State PHL to a local commissioner of health and/or a local department of health would be deemed to refer to the newly formed Department or its Commissioner, as appropriate. The Commissioner was to be a County Health Commissioner within the meaning of Article 3, Title III, of the PHL (SCC § C9-1, § C9-2; L.L No. 25 of 1973). Subsequently, vector control activities were the responsibility of the Division of Public Health in SCDHS.

However, in 1992, amendments to Sections C8-2 and C8-4 of the SCC established the SCVC as part of the Suffolk County Department of Public Works (SCDPW) and authorized the Division to "use every means feasible and practical" to suppress mosquitoes and other arthropods (SCC § C8-2, § C8-4; L.L. No. 16 of 1992). That Local Law also noted as follows:

> (A)lthough the authority for the county to establish a vector control program is contained within the New York State PHL, this law does not mandate that vector control activities be performed under the auspices of the local Health Department. However, in the event that an arthropod-borne disease is found to constitute a major public health threat, the DHS shall directly supervise vector control (L.L. No. 16 of 1992, Section1).

SCVC is responsible for controlling mosquito infestations that are of public health importance, pursuant to the powers granted to the County under the PHL. In the event of a vector control

emergency, "as defined" by the Commissioner of Health Services, the direct supervision of vector control shall be by the DHS (SCC § C8-2[Y], L.L. No. 16 of 1992).

The SCDHS is responsible for monitoring and prevention of human diseases, including those borne by mosquitoes such as WNV and EEE. SCDHS monitors the blood supply, handles reports of WNV and EEE infected birds and horses, and responds to health emergencies through its Division of Public Health. In the event that an arthropod-borne disease is found to constitute a major public health threat, the vector control program would be under the control of SCDHS (SCC, § C8-2[y], L. L. No. 16 of 1992). SCDHS, Division of Environmental Quality, through its Office of Ecology, manages a number of water quality and restoration programs that involve wetlands managed by the Division of Vector Control. The Office of Ecology is the program director for the Peconic Estuary Program, and is the major County participant in the South Shore Estuary Reserve and the Long Island Sound Study.

According to the SCC, SCVC shall have

> charge and supervision for vector control throughout the County of Suffolk. The Department shall have the power and authority to enter without hindrance upon any or all lands within the county for the purpose of performing acts which in its opinion are necessary and proper for the elimination of mosquitoes and other arthropods, provided that such measures are not injurious to wildlife. In the event of a vector control emergency, as defined by the Commissioner of Health Services, the direct supervision of the vector control shall be by the Department of Health Services." (SCC § C8-2(Y).

The charter also specifies the powers of SCVC, and relates its responsibilities. The Division of Vector Control

> shall use every means feasible and practical to suppress mosquitoes, ticks and other arthropods which are vectors of human disease requiring public action for their control. In carrying out its responsibility hereunder, the Division shall have the power and authority to enter without hindrance upon any or all lands within the county for the purpose of draining or treating the same and to perform all other acts which, in its opinion and judgment, may be necessary and proper for the elimination of mosquitoes and other arthropods, but such measures shall not be injurious to wildlife (SCC § C8-4(B) (1))

The responsibilities listed for SCVC include submitting an Annual Plan of Work to the Legislature each year, and various public noticing requirements. These include different kinds of notices for truck and aerial applications, and also for when there has been a declared health emergency, and when there is not such an emergency.

2. Public Education and Outreach

Responsible mosquito management, conducted according to IPM, involves a hierarchical approach to identifying and addressing problems. The hierarchy is:

- Scientific surveillance
- Source reduction (including water management)
- Larval control
- Adult controls

The use of biological controls (biocontrols) is usually an important element. A key area tying together all of these facets of mosquito management is public education and outreach. In a sense, public education is part of the control hierarchy, as an educated public can take steps to eliminate mosquito breeding areas around the home and protect themselves effectively from mosquito bites and the effects associated with the bites. However, nowhere has it been possible to achieve compliance rates for personal protection so as to eliminate the need for organized vector control to ensure public health and welfare.

2.1 Public Education

Public education is a key element of the Management Plan. Public education can:

- help people avoid mosquitoes and mosquito-borne disease
- raise public awareness of the value of good housekeeping
- ensure the public cooperation essential for Vector Control's operation
- provide justification for the actions taken by the County on behalf of its citizenry to control mosquitoes and mosquito-borne disease, and,
- avoid public demand for more pesticide applications than are truly necessary, out of excessive concern over mosquito-borne disease.

SCDHS is primarily responsible for public education on mosquitoes and mosquito-borne disease. It has greatly expanded its role in educating the public about the public health importance of mosquitoes and in developing the means to keep the public informed, should control measures be necessary.

WNV vectors such as *Cx. pipiens* and *Oc. japonicus* often breed in artificial containers, clogged rain gutters, birdbaths, and other inaccessible places found around the house. *Cx. pipiens*, in particular, prefers polluted water for breeding. This means that maintaining items such as birdbaths, or emptying containers in the yard, can have substantial impacts on risks associated with disease transmission. The County will promote information on personal protection and avoidance by distributing brochures and giving presentations on its "Dump the Water" and "Fight the Bite" programs. Additionally, the Long-Term Plan Citizens Advisory Committee created a new outreach newspaper insert titled, "Mosquito Control and Prevention at Home." The County anticipates using this resource, as well.

SCDHS began its annual Dump the Water Campaign in 2000. It was created by legislation, through the Suffolk County Legislature. Every year in January, elementary students in Suffolk County participate in a poster contest hosted by the members of the Legislature. The winning poster is used as the cover for the Dump the Water public education pamphlet. The Dump the Water pamphlet includes information on how WNV is transmitted and what the public can do to eliminate mosquito-breeding sites around their homes. The pamphlet encourages the public to educate their neighbors and local business owners, in addition to getting involved in organizations that participate in clean-up drives. It also lists contact numbers and website addresses for SCDHS, SCVC, and NYSDOH for further information. The Dump the Water brochure will be fine-tuned so that it does not appear to suggest that residents may remove aquatic vegetation without first obtaining any required State permits.

The "Fight the Bite" pamphlet includes information on:

- facts about mosquito species
- where they live and breed
- symptoms of WNV

- who is most at risk of contracting WNV
- when mosquitoes are most active
- what can be done around the house to diminish mosquito-breeding sites

An illustration is included to demonstrate where typical mosquito breeding sites can be found around the home. The brochure also provides examples on what to do to protect oneself from mosquitoes, how to properly use products containing the mosquito repellant DEET, and what to do after spotting a dead crow. As with the SCDHS brochure, the New York State publication also includes contact information, although this information is for statewide offices concerned with mosquito control.

In addition to the SCDHS efforts, SCVC offers public assistance to help homeowners and other property owners and land managers who have mosquito problems, by visiting the property and removing breeding areas. If the homeowner is not available during the site inspection, SCVC ground crews hang tags on the front door knob. The door hanger describes the reason for the inspection and lists any work done. It also provides basic information about mosquito control. The tag gives contact telephone numbers, and directs the homeowner to the SCVC website for more information.

Each year during the off-season, prior to the development of the coming year's brochure, field personnel from SCVC should interact with the health educators from SCDHS. This will allow transfer of information from the field to the educators regarding the kinds of persistent problems that are not being reduced through current education programs. In addition, field crews will be made aware of the current focus and ranges of materials used by the educators, which should enhance the field crews' education efforts, as well.

Another way in which SCDHS could improve public outreach is to participate in "Mosquito Awareness Week," which is an American Mosquito Control Association (AMCA) sponsored program that takes place at the start of the summer season. This program provides mosquito control professionals with a time frame that can be devoted to focusing the public's attention on the services SCVC provides. An electronic flyer is sent to AMCA members and mosquito control supervisors. The flyer includes a template for press releases to local newspapers, along

with ideas for content. It also offers suggestions on ways to distribute mosquito control information to various media outlets, such as serving as a guest expert on a local radio show. Mosquito control supervisors are reminded to have technicians mention and emphasize the positive changes and environmentally friendly methods used in mosquito control.

Since the County's human population is becoming more diverse, publications in upcoming years may wish to draw upon New York City's experience and capabilities for outreach to immigrant groups. The New York program produced outreach materials in 17 languages; this available material may decrease the learning curve should the County determine that other languages, such as Spanish, need to be added to its educational arsenal.

This educational program should be expanded to include a tire disposal component. Outside review of the existing County program found the lack of a tire management component to be a major deficit. Tires are a major breeding opportunity for mosquitoes because they retain water so well. The mosquito that breeds most readily in tires, *Oc. japonicus*, has also been identified as a major relative risk factor for WNV, despite its relatively low numbers in the County, because it is a very efficient vector of WNV. It has the great potential to transmit the disease to people should it become infected, due to its propensity for feeding on mammals. The County should undertake an education program to persuade citizens not to inappropriately discard tires, but to manage them properly. Tires should not be stored out of doors. The County should conduct internal outreach so that Departments such as Parks and Public Works, in the course of other maintenance activities, understand the importance of removing littered tires when encountered. Although the Towns are the level of government responsible for zoning and waste management in the County, the County should determine if it can provide useful resources to allow Towns to address tire stockpile issues. One notorious stockpile is in Smithtown (Old Northport Road), where millions of tires were buried to reduce risks associated with fire. This also eliminates mosquito habitat, but unless this site and others like it are monitored and managed, they hold the potential of becoming serious health risk loci.

Water use issues in farm areas are often of concern, especially on the East End where water quantity may become a more pressing issue as populations rise. Irrigation can cause ponding on fields which generates mosquito breeding habitat. SCVC already maintains certain water

management structures in agricultural areas to drain standing water. Targeted education through Cornell Cooperative Extension can reach this audience efficiently, and reinforce the already delivered message regarding best practices for irrigation conditions.

Another missing element in the current County outreach program is something undertaken in Westchester County. This is targeted outreach to commercial property owners and private homeowner associations to ensure that private storm water systems are properly maintained. In this instance, a well-worded insert in tax bills (or separate mailing utilizing County property data bases), identifying benefits to the County that include decreased flood impacts, improved public health, and avoidance of a label as a public health nuisance, may encourage neglected maintenance to be undertaken.

Similarly, SCVC, through SCDPW, needs to raise awareness in the County and in other municipal highway offices, that poor maintenance of catch basins and other storm water systems not only exacerbates flooding problems, but threatens public health. These underground facilities are prime *Cx. pipiens* habitat. Although it is uncertain how much WNV is actually transmitted by *Cx. pipiens*, several cases of WNV in the County have occurred where trapping has found *Cx pipiens* almost exclusively. It is clearly an important prime amplification vector for the disease. Any reduction in the numbers and range of this mosquito, which tends not to fly too far from its habitat, has immediate implications for the risks faced by people in the immediate vicinity of the structure that has been maintained.

Areas that have historically experienced vector control adulticide treatments (roughly speaking, Babylon, Islip, and Brookhaven south of Sunrise Highway) should receive augmented, targeted education efforts. These efforts will focus on personal protections steps to minimize negative impacts from mosquitoes, such as wearing long-sleeved shirts and long pants, using repellents, and avoiding outdoor activities during peak mosquito times. In addition, the Commissioner of SCDHS will identify pertinent actions that residents should consider to reduce exposure to and impacts from any adulticide applications. Currently, the public notice for adulticide applications includes the following language:

> Steps you should take: Children and pregnant women should take care to avoid exposure when practical. If possible, remain inside or avoid the area whenever spraying takes place and for about 30 minutes after spraying. Close windows and

> doors and turn off air-conditioning units or close their vents to circulate indoor air before spraying begins. Windows and air-conditioning vents can be reopened about 30 minutes after spraying. If you come in contact with pesticide spray, protect your eyes. If you get pesticide spray in your eyes, immediately rinse them with water. Wash exposed skin. Wash clothes that come in direct contact with spray separately from other laundry. Consult your health care provider if you think you are experiencing health effects from spraying.
>
> Steps you may wish to take: Cover outdoor tables and play equipment before spraying or wash them off with detergent and water if exposed to pesticides during spraying. Bring laundry and small toys inside before spraying begins (wash with detergent and water if exposed to pesticides during spraying). Bring pet food and water dishes inside, and cover ornamental fishponds to avoid direct exposure.

Presentations at schools, to civic organizations, and other interested groups, and news releases to local newspapers will all be used to specially inform these citizens who are more likely to be exposed to mosquito bites and adulticide applications than other people living in Suffolk County. Targeted education and outreach efforts will similarly be undertaken if the FINS-specific mosquito control plan includes adulticide applications for vector control purposes.

County health educators will look to increase opportunities to speak at schools and civic organizations. These may be very effective audiences to result in behavioral changes, as school children are well-known for carrying lessons home to parents. Civic organizations in areas where mosquito problems are more common seem much more likely to be receptive to messages that explain how to reduce risks from disease and otherwise limit exposure to and ill-effects from mosquitoes.

In addition, the County will also seek to use different means of outreach to County residents, as resources allow. Public Service Announcements (PSAs), whether for radio or TV, can be effective in reaching audiences that may not seek out brochures or attend community meetings. Creating professional quality PSAs requires extensive resources that are not currently available to the County. However, SCDHS health educators will be tasked with being creative and innovative in finding means to use these promising outreach tools.

The County websites for SCVC and SCDHS provide current information about upcoming spray events and general work of SCVC, and information about what the public can do for protection

from mosquitoes, and to help combat mosquitoes around their homes. The website also describes the various methods and products used by SCVC for mosquito control. Information regarding the dates and events taking place during "Mosquito Awareness Week" will also be made available on the SCDHS website with links to each of the brochures the used in the public education program.

Another recommendation for public outreach is to post efficacy reports on the SCVC website at the beginning, middle and end of the season. These reports will summarize the results of mosquito control efforts that were measured before, during and after aerial spray events. Reporting efficacy to the public will emphasize how SCVC operations are improving the quality of life in their community and throughout Suffolk County. Public support for vector control operations will aid the County in justifying the need for the formation of the new Mosquito Surveillance and Control unit, which will perform quality assurance and quality control functions.

It has been shown that people who adopt personal protection measures (wearing appropriate clothing and/or repellents, or who avoid exposure to biting mosquitoes) have significantly lower infection rates compared to those who do not adopt such actions. Therefore, it is clear that public health benefits can be reaped through an active, engaged, and widespread public education effort. Although more attention is paid to such outreach during mosquito seasons, the County is urged to keep this aspect of the program a year-round priority, to the point where all residents and visitors to the County are aware of the measures that can be made in order to increase personal protection from vector-borne disease. Indeed, given the prevalence of Lyme disease in the County, perhaps a joint arthropod-borne disease effort might reap benefits for those in the County who spend time outdoors during milder weather.

Of course, public outreach efforts will be increased as risks associated with mosquito-borne diseases increase through the summer. SCDHS will increase its efforts to introduce mosquito avoidance techniques into local media through press releases and similar tools. As appropriate, the Commissioner of SCDHS and/or the County Executive will use the bully pulpit associated with their positions to urge residents to take reasonable precautions to avoid disease exposure. Where identified virus threats have been found, as has been the case at Blydenburgh County Park

in 2004 and 2005, specific areas may receive extra notifications and signage to warn users to be extra vigilant to reduce health risks. Special signs may also be posted at access points to natural areas on Fire Island, where non-intervention policies supported by NPS mean that mosquito populations are often extremely high. FINS and the County will develop appropriate procedures that meet County needs to reduce health risks and NPS requirements regarding avoidance of interference in natural processes; this may include more explicit written notifications regarding risks from disease or procedures to minimize visitations if risk factors exceed certain levels.

The Long-Term Plan web site material will be incorporated into background information areas on the County health department and SCVC websites. The Triennial Program Update Reports will also be used as outreach materials.

2.2 No-Spray Registry

Suffolk County adopted a law in 2001 which resulted in the creation of the "no-spray registry." The law requires SCVC to "make a good faith effort" to exclude each property by stopping adulticide spraying from trucks within 150 feet on either side of the registrant's property. Operationally, this translates to sprayers being shut off on the street in front of the registered property and 150 feet on either side of the property. Citizens can sign up for this registry via the SCDPW website, or by calling the SCVC directly. This registry represents an effort to balance the desires of those residents who want control of adult mosquitoes with those who are more concerned about the potential effects of pesticides, however small the risk. In 2005, a little less than 900 properties were registered. Many of these, as it happens, are located in areas where serious infestations were rare. For this reason, the registry has thus far had little effect on control operations. It is recommended that the registry's existence be included in public education presentations and printed in educational brochures. This will allow residents without Internet access to join the list, if desired.

When control is required to deal with a public health emergency, the Commissioner of SCDHS can override the list. Even then, efforts are made to telephone list members prior to applications in their area.

In addition to this legally required registry, the SCVC maintains special listings of beekeepers and organic farms. Beekeepers are generally avoided or notified before treatments so that they can protect their hives. Because the commonly used SCVC adulticides are not registered for croplands, organic farms and all other croplands are excluded from spray areas to ensure label compliance. Organic farms are specially called out because many are small and in otherwise residential areas.

2.3 Notification

In 2000, the County passed new laws to improve required public notification for adult mosquito control. As a result, there is now an increased use of the media and extensive outreach to local officials when any such actions are considered. The SCDHS web site is used to post maps and will be used to post spray schedules. In addition, a list serve feature will be installed on the SCDHS website to allow citizens the choice to automatically be informed of spray events. For each adulticide application, over 150 faxes are sent to various officials and other interested parties. Newsday and News12 post spray schedules and maps and "No Spray" members are telephoned. Notifications are also broadcast over several local radio stations, posted on a call-in hotline, and on orange signs at the entrance of parks where applications are scheduled. The Suffolk County Board of Cooperative Educational Services (BOCES) is also notified, and BOCES, in turn, notifies schools.

It is important to recognize that adulticide applications have well-defined acceptable weather conditions. The need to inform the public will need to be balanced with the need to conduct operations promptly, within weather windows and before the problem spreads and more acreage may need treatment. It is not appropriate to provide more than 24 hours notice in most cases, because beyond that time, weather forecasts do not have the necessary reliability to schedule the application events. Attempts to provide more than 24 hour notice can result in many spray operations being announced but then cancelled, which can be very confusing to the public.

In addition to these formal outreach operations, the Long-Term Plan envisions continuing its Citizens Advisory Committee as a means of having on-going dialog with involved members of the public. This Committee has served an important role in the course of developing the Plan, and has routed useful and important information and viewpoints to planners and researchers.

SCVC has also had the opportunity to renew contacts with members of various state, federal, and local agencies and governments, and certain interested non-governmental organizations, through the Long-Term Plan. The Wetlands Subcommittee of the Technical Advisory Committee has been a key venue for these efforts. The Long-Term Plan requires that similar kinds of communications continue in order to achieve important aspects of the proposed Plan; the Wetlands Stewardship Committee will be an important group for continuing discussions between SCVC and other important and involved organizations in mosquito control (especially in wetlands management).

2.4 Website

Information on WNV, the Long-Term Plan process, pesticide application notification and a "No Spray" registry are included on the County mosquito control website. However, only the pesticide spray schedule and "No Spray" registry are regularly updated. Annual reports from SCDHS are also available on the website. These reports are not current and focus on SCDHS operations, such as Emergency Medical Services and Mental Health Services, versus vector control program information. Both deficiencies stem from resource allocation limitations.

The website needs to be updated, and a means of regularly posting new and relevant information there must be established. The results of efficacy testing, for example, and the various annual and other reports that will be produced on a regular basis as a result of the Long-Term Plan should be made available to the public.

3. Surveillance

3.1 Background

The goals of a sound surveillance program are to monitor the distribution and abundance of larval and adult mosquitoes and the prevalence of mosquito-borne diseases. This is accomplished by a variety of mechanisms, including trapping mosquitoes, monitoring their breeding, analyzing them for evidence of viral activity, and monitoring other species that may have become infected by mosquito-borne disease. Results from monitoring are used in progressive mosquito control efforts to determine appropriate interventions in order to reduce impacts to human health and public welfare, minimizing the risks and impacts associated with the intervention chosen. Sufficient monitoring efforts lead to an adaptive response program, as interventions can be tailored to achieve the desired impact on the targeted mosquito populations — that is to say, effective monitoring leads to gauging the effect of interventions, and so tuning the level of intervention to meet changing conditions.

Larval surveys can determine the location, species, and population densities of mosquito larvae for predictions of adult emergence and for gauging required control measures. They are also utilized to assess the effectiveness of larval control measures. This requires examining aquatic habitats for the presence of the larvae, and colleting samples for speciation (in the field or back at a laboratory).

The three most commonly used adult mosquito traps are the New Jersey light trap, the CDC (Centers for Disease Control and Prevention) light trap, and the CDC gravid trap. The New Jersey light trap attracts a variety of mosquito species and is especially useful for monitoring long-term changes in adult mosquito population density and species composition. Because the New Jersey light trap kills mosquitoes, it cannot be used to monitor virus activity. Some species of interest are not attracted to this type of light trap. Human biting mosquitoes (including *Ochlerotatus sollicitans* and *Culex* spp.) are sampled with CDC traps to provide additional viral surveillance. CDC light traps utilize CO_2 as an additional attractant. Gravid traps are used to sample for egg-bearing (gravid) mosquitoes. Gravid traps utilize what is described as "polluted" water (organic water, often odiferous that specifically is attractive to *Cx. pipiens*). Both kinds of CDC traps provide live mosquitoes, which is a prerequisite for viral assays.

Another critical component of a sound monitoring program for the Long Island region is surveillance for the presence of EEE and WNV. CDC light and gravid traps are used at locations chosen for their history of viral activity to trap the mosquito vectors of these diseases.

Disease surveillance also includes reports from veterinarians and doctors regarding disease outbreaks. Because birds are intimately involved in many arbovirus transmission cycles, testing of dead or captured wild birds and monitoring of sentinel domestic flocks is also widely used to determine if viruses are becoming a human health concern.

Surveillance is intended, according to guidelines from both CDC and NYSDOH, to provide local officials with appropriate information to make informed decisions regarding disease risk. This information is essential in order that responses to risks are consistent to the level of health threat posed by the mosquito populations. These guidelines describe increasing degrees of surveillance and control as the risk of disease transmission increases.

3.2 Larval Mosquito Populations

Larval surveillance provides information on expected adult mosquito density and areas where source elimination or larvicide efforts should be targeted. Teams of inspectors, consisting of three foremen with 11 field crews that each consist of two equipment operators or laborers, will continue to be assigned to geographic areas of the County to guarantee complete coverage of potential breeding habitats on a regular basis. The number of field crews assigned to each geographic area is dependent upon the number of wetlands located within each area. The names of the geographic areas and the number of field crews assigned to each area are listed in Table 3. These areas are designated as:

- south shore west, which includes Babylon, Islip, and southwestern Brookhaven
- south shore east, which includes southeastern Brookhaven, Southampton, and East Hampton
- north shore west, which covers Huntington and Smithtown
- north shore east, which includes northern Brookhaven, Riverhead, and Southold

Inspectors from the north shore west areas also assist with surveying salt marshes in south shore west areas (such as Gardiners Park and Heckscher State Park).

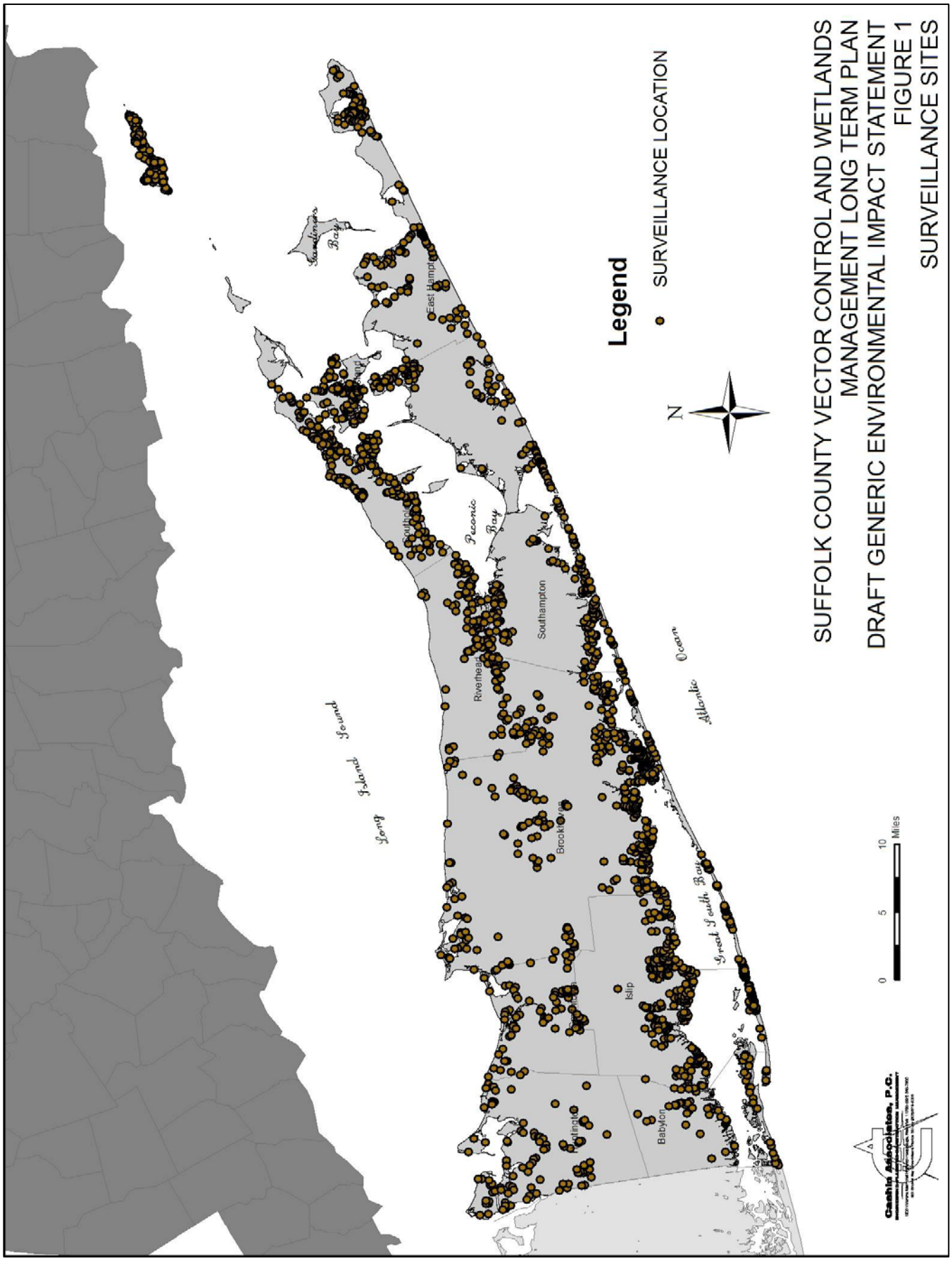
Table 3. Geographic designations for larval surveillance

Geographic Area	**# of field crews**
south shore west	3
south shore east	4
north shore west	2
north shore east	2

Inspectors obtain samples from larval breeding areas, such as wetlands, primarily using a technique known as dipping. Dipping is performed using a cup on a long dowel handle to collect a small amount of water. The presence of mosquito larvae in the water indicates breeding activity. Inspectors will quantify larval surveillance results in the field by counting the number of larvae per dip. They will also determine which of four larval stages are present. Dipping is a time-honored mosquito surveillance tool, and it is possible to count the larvae present in any sample. This suggests that the technique is quantifiable. However, dipping results vary from sampler to sampler, and are generally not replicable. Dipping is also best accomplished in a non-random fashion, in that larvae should be actively sought. Larvae may be concentrated at one part of the habitat by wind, or may be sheltering among emergent vegetation, or may be avoiding shadows or other cues; almost certainly, they will not be evenly (or even randomly) distributed. The act of dipping itself will disturb larvae that are present, and so repeated dips are likely to catch fewer individuals. In addition, concentration (or not) of the larvae may skew results. There may be more larvae per dip in a potholed high marsh compared to samples taken from a panne environment, but the greater amount of water in the panne may result in a higher absolute number of larvae per acre of marsh there than in the potholed area. Therefore, although the number of larvae can (and in many cases, routinely is) counted and tracked, the data so produced may have less meaning than it seems to. Higher values may not represent greater breeding potential, nor may low numbers mean that few adult mosquitoes will be produced. Some consistency in results may be fostered by having the same personnel sample the same sites, and to trust that these staffers conduct the sampling similarly each time. Dipping most reliably reports presence or larvae; scrupulous surveillance by inspectors implies that no detections of larvae in the dips means no to little mosquito breeding.

At times, sampling methods other than dipping will need to be employed to determine if specific species are present. Tires, for instance, are often sampled using aspirators. Various means of pulling water out of cavities are used to sample in tree crypts for *Cs. melanura.* *Cq. perturbans* larvae attach themselves to underwater plants, and need to be pulled from this attachment. Catch basin sampling has led to specialized equipment. Often, aquarium nets are attached to telescoping poles, and then the nets are rinsed to wash the larvae into a bucket for further processing.

SCVC has identified over 2,000 breeding points throughout the County (see Figure 1). These are areas where problem mosquito populations have re-occurred. The sites range from small, intermittent freshwater wetlands to salt marshes that can be several hundred acres in size. Each breeding location has been assigned a unique identifier, composed of letters (for the Town) and numbers (so that BH-112 would be in Brookhaven). Each sampling point has also been mapped using GPS, and to further encourage consistent sampling, it tends to be monitored by the same inspector team.


SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS
MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 1
SURVEILLANCE SITES
Legend
SURVEILLANCE LOCATION
N
0
5
10
Miles
Long Island Sound
Peconic Bay
Great South Bay
Atlantic Ocean
East Hampton
Southampton
Riverhead
Brookhaven
Islip
Babylon
Huntington
Cashin Associates, P.C.

Breeding locations are monitored on different schedules according to the type of mosquito problem that is usually associated with the particular site. The highest priority surveillance locations are the salt marshes that are candidates for aerial larviciding. These marshes are monitored every Monday for breeding activities. Additional monitoring can be required if environmental conditions (unusually high tides or heavy rainfall) suggest that mosquitoes may hatch after Monday and temperatures indicate they will develop quickly. Then inspectors may be requested to revisit the sites later in the week.

Each field crew also is assigned a route of smaller salt marshes and fresh water sites that also tend to breed fairly regularly. These sites are monitored on a 10 day to two week cycle. SCVC supervisors may authorize overtime to ensure routes are completely monitored more expeditiously if environmental conditions (again, higher tides or heavy rainfalls) indicate that breeding is more likely to be occurring.

Finally, there are certain locations that only support breeding under particular environmental conditions. The field crews develop experience regarding some of these locations, and only visit them when the requisite trigger has occurred. These may be breeding sites very high in a marsh, or where heavier rainfalls lead to pooled water.

Note that higher tides and/or heavy rains often lead to widespread breeding. These events can result in a need to investigate nearly all breeding sites throughout particular environmental settings and lead to manpower stresses (which are usually addressed through authorized overtime, but may require assignments of additional staff from other important tasks).

Salt marshes have specialized monitoring requirements. It is important to establish set monitoring points on a marsh to be consistent. These should be established in the high marsh, as the irregularly flooded portion of the marsh is the portion that breeds mosquitoes. In addition, it is very important, either through using multiple locations in each marsh, or through field crew observations, to record the extent of the flooding in the high marsh. This is because the size of the brood associated with a flood depends on the extent of the marsh that was inundated. Wetted eggs concentrate into the retained pockets of water and develop into larvae. Therefore, it is also important to judge the stage of the larvae, and the speed with which the marsh is drying down. Successful breeding for mosquitoes is often a race between organism development and

disappearance of the aqueous habitat; both are accelerated in warmer temperatures. Surveillance for salt marsh mosquito larvae therefore requires determination of presence/absence of larvae in the marsh, the extent of the initiating tidal inundation of larvae are found, the dominant stage of the larvae, and the remaining water on the marsh. The latter two parameters, when combined with apparent development rates (which are temperature dependent) and evaporation forecasts (also largely temperature dependent), can result in a forecast of whether the brood will successfully hatch or not (in the absence of intervention).

Brackish and fresh tidal marshes also need to be sampled, as these are important habitats for other pestiferous mosquitoes in the County (such as *Ae. vexans*). Similar kinds of considerations hold for these environments.

In addition to the 15,000 catch basins monitored in 2005, two to three times that number (30,000 to 45,000) of catch basins should be monitored beginning in 2006. Factors that will be used in selecting additional basins for monitoring include:

- basins with a history of viral activity in the surrounding area
- the age of the system (older basins tend to support more breeding, most commonly because they have not been well maintained)
- systems where maintenance may have been deferred (see just above)
- basins located at the terminal end of drainage systems or in low lying areas (these tend to hold water for longer periods of time)

The County has not yet created a GIS map of all of its storm water systems. Expansion of the catch basin surveillance network will be addressed at first based on inspector familiarity with areas under current surveillance, according to the parameters listed just above. More systematic approaches to this process should become available with better information resources.

Expanding the geographical extent of catch basin surveillance will allow the County to monitor breeding over a larger area, decreasing the potential of disease transmission. The catch basins will be sampled beginning in late May or early June for the presence of larvae. Basins will be

revisited and re-sampled, as resources allow, preferentially during the middle (July) and end of the season (September), to monitor the development of any additional breeding activity and the efficacy of control efforts. Although some jurisdictions have quantified breeding in basins, presence/ absence of larvae is a more conservative indicator of the potential need for treatment. This is because sampling techniques are predicated on determining if breeding is occurring, but not necessarily to ensure each sampling effort is commensurate with each other. The best means of sampling a catch basin is to use an aquarium net on a telescoping pole. The net is swirled through any standing water in the basin as can best be managed, given the alignment of any manhole grating. The aquarium netting is then rinsed into a bucket, and larval samples collected into appropriate containers for further analysis at the laboratory.

It is recommended that SCVC increase the number of recharge basins that are sampled and visited in a manner similar to the one described above for catch basins. Recharge basin larval sampling is done using standard dipping techniques, with an emphasis on sampling in any emergent vegetation.

The field crews will examine and determine the larval stages present in samples in the field. Training in identification allows for reliable staging of most larvae. This process is somewhat simplified by habitat specialization by many species, so that *Oc. sollicitans* is the dominant summer mosquito in salt marshes, and *Cx. pipiens* is the typical mosquito found in catch basins. Collected larvae will be stored in glass sample jars. The samples will then be transported to the laboratory for species identification by an entomologist.

3.3 Adult Mosquito Populations

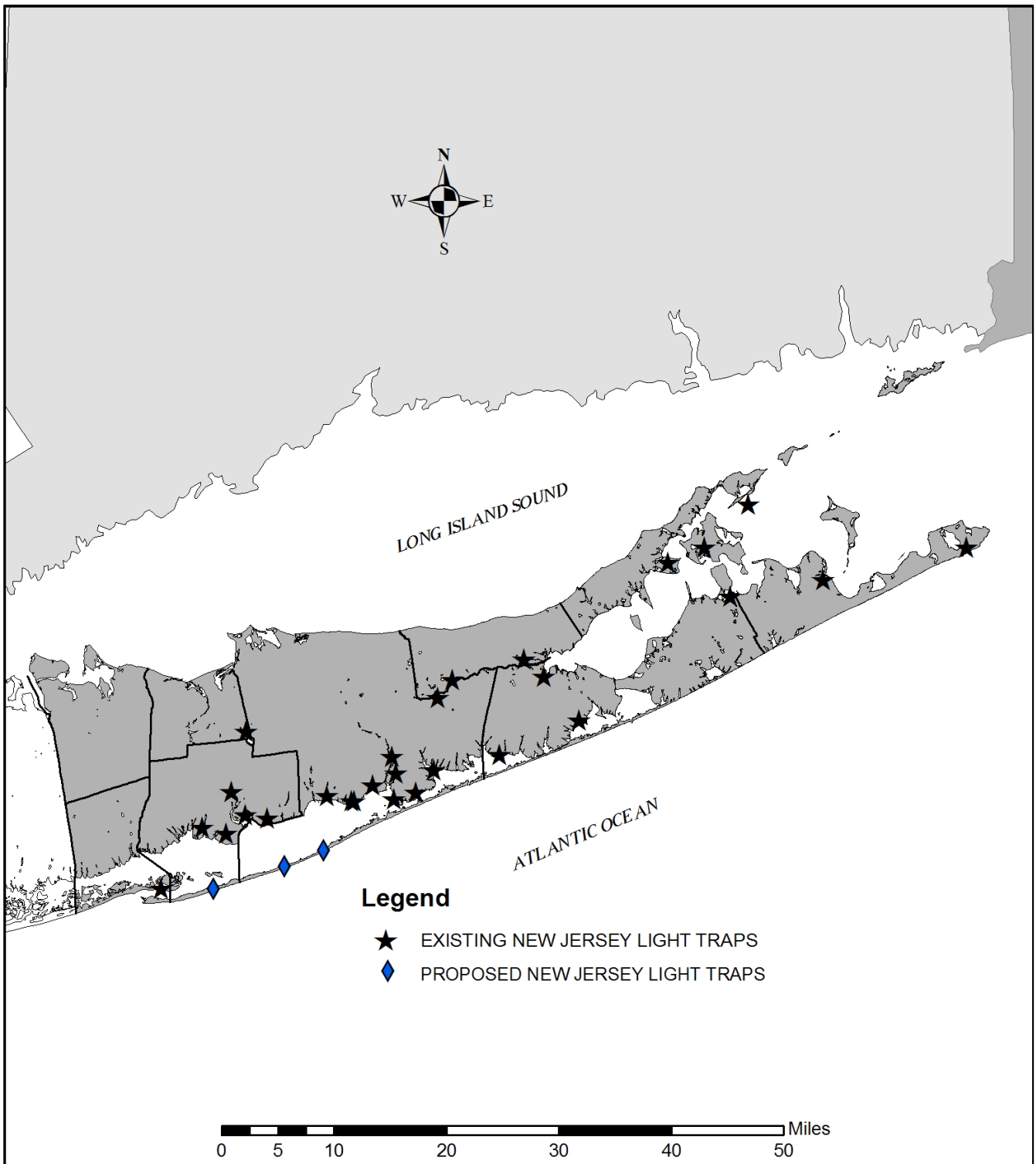
Surveillance of adult mosquito populations is necessary for locating infestations that impact quality of life and/or public health, directing control efforts and evaluating the effectiveness of those efforts. Populations of adult mosquitoes are monitored using New Jersey light traps and CDC light traps. Population surveillance has some role in disease surveillance and disease risk management, but generally is considered to be a separate activity.

A New Jersey light trap consists of a light bulb placed above a metal cylinder with a fan fitted to the top of the cylinder. The fan draws mosquitoes that are attracted to the light into the cylinder, where they become trapped and die from dehydration. Traps operate continually, i.e., seven days

per week, in an effort to sample all mosquito species that may be present in a given area. Dead specimens in the traps are collected up to three times a week. Statistics are generally reported on a weekly basis.

New Jersey traps are set at fixed locations. They are used to directly measure the abundance of mosquitoes in an area. The data can be used to evaluate the effectiveness of the local larval control program. When a New Jersey trap is fortuitously placed, it can also be used to evaluate the effectiveness of an adulticide event. New Jersey light traps require power sources of 110 volts, which limits mobility. New Jersey traps are typically placed in a residential area with a history of mosquito problems to provide long term monitoring of the area. Because of their fixed locations, New Jersey traps are generally not optimally placed to monitor particular problem sites. They are usually considered to provide surveillance for a general area. This is reflected in their nomenclature, as they are generally named for surrounding hamlets (e.g., "the Oakdale New Jersey trap"). New Jersey traps are often maintained in the same location for years or even decades, providing valuable information on long term changes in mosquito populations.

The County currently has 27 New Jersey light traps at fixed locations throughout the County. The network is focused on the South Shore, and salt marsh mosquito problem areas. Traps are also set to monitor other coastal areas, and also to measure impacts from fresh water mosquitoes (see Figure 2). The County has currently proposed augmenting this network with three additional trap locations on Fire Island (subject to completion of a separate Fire Island planning process), and potentially some others to measure ambient mosquito counts in areas with no control.



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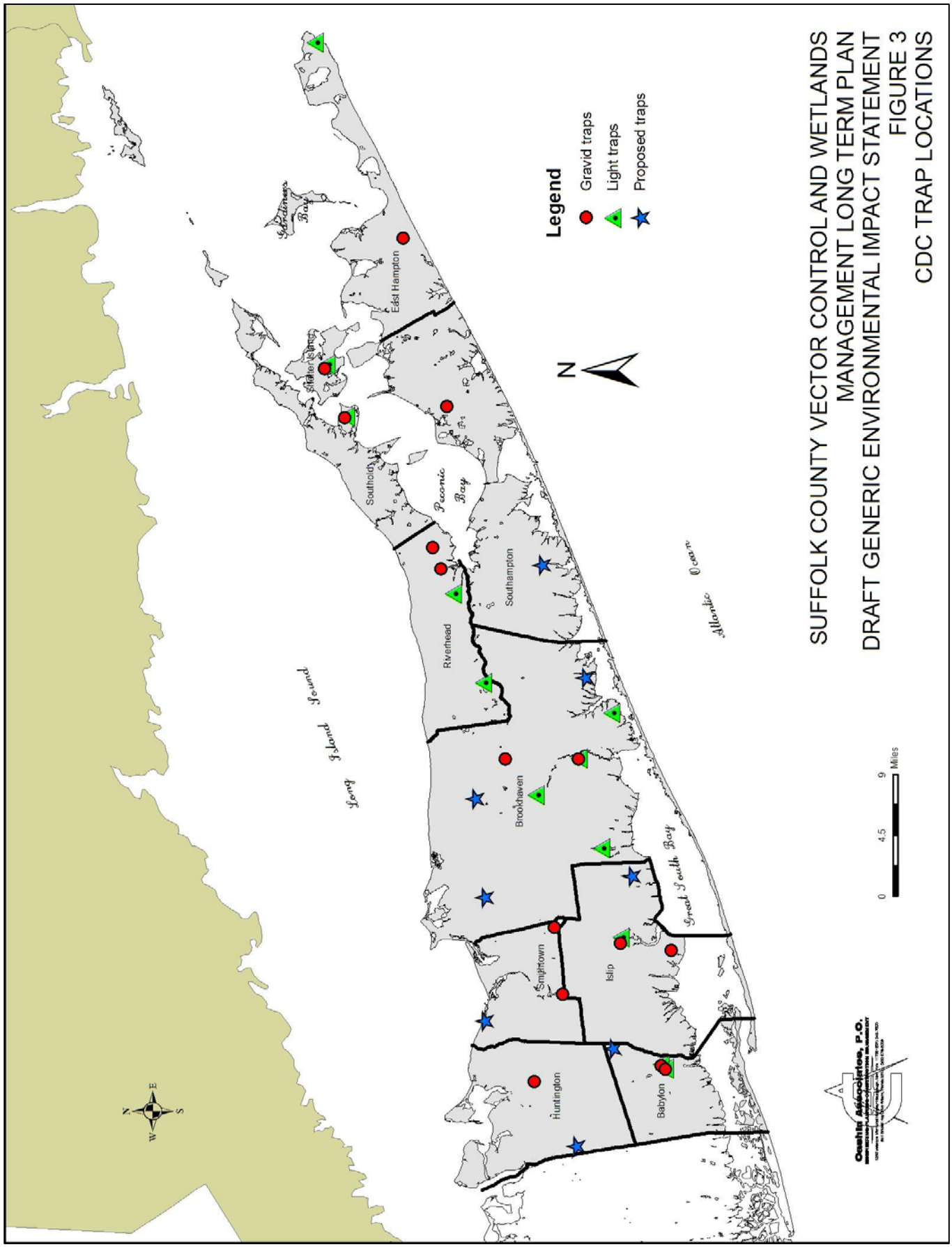
SUFFOLK COUNTY VECTOR CONTROL AND
WETLANDS MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL
IMPACT STATEMENT
FIGURE 2
NEW JERSEY LIGHT TRAP LOCATIONS

CDC light traps differ from New Jersey light traps in that they are baited with dry ice (frozen carbon dioxide [CO_2]) or some other CO_2 source. CDC light traps are more portable and are commonly used to collect human-biting species and *Cs. melanura*. The dry ice placed in the trap releases CO_2 gas as it sublimates. This mimics the gas contained in the exhalations of active animals, thereby attracting mosquitoes, and increasing the catch compared to New Jersey traps. Mosquitoes are captured live. These traps are set in the evening and collected in the morning. CDC light trap samples analyzed for population purposes do not need to be preserved following collection (those for disease surveillance need to be cold preserved to keep the mosquitoes alive).

CDC light traps can be set at fixed locations. For example, Fire Island National Seashore (FINS) uses CDC traps for its population sampling. SCVC has tried to use New Jersey traps for routine population sampling because New Jersey traps do not require as much effort to set and monitor. SCVC has typically set CDC traps for population monitoring when special problems have been identified (and for disease monitoring purposes). CDC light traps can be set in areas where the volume of complaints increases, or where there are other indications that a mosquito biting problem will not be detected by the fixed New Jersey trap network.

CDC gravid traps are also used in the surveillance program. Gravid traps are generally not suitable for population monitoring purposes, as they tend to be more selective in the kinds of mosquitoes they trap. Currently, the ABDL uses a fixed network of 27 CDC traps (12 light and 15 gravid traps), and augments this fixed network with as-needed locations, based on pathogen detections. The ABDL will expand the network to 35 initial traps in 2006, and continue to expand as resources allow. The eight additional initial traps will be a mix of gravid and light traps, to be determined by the director of the ABDL (gravid traps tend to be easier to manage, but light traps provide greater amounts of information). Figure 3 illustrates the current and proposed network of CDC traps.


SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS
MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 3
CDC TRAP LOCATIONS
Legend
Gravid traps
Light traps
Proposed traps
N
East Hampton
Southold
Riverhead
Southampton
Brookhaven
Islip
Smithtown
Huntington
Babylon
Peconic Bay
Great South Bay
Atlantic Ocean
Long Island Sound
0
4.5
9
Miles
Cashin Associates, P.C.

The County currently focuses its population trapping efforts on areas with consistent mosquito problems. Due to resource limitations, several areas with a history of biting complaints are currently not monitored for the presence of mosquito larvae and lack traps to regularly monitor adult mosquito populations. Examples include areas along the north shore and Blue Point along the south shore, and some areas that are located some distance away from Yaphank, such as Shelter Island, Fishers Island, and Fire Island.

A means of addressing the travel issue associated with sample retrieval is to establish identification stations. The stations would consist of a single room within an existing municipal building, located relatively close to the aforementioned underserved areas, that is equipped so as to allow field technicians to identify mosquitoes to the species level. This would eliminate the need for the technician to return the samples to the laboratory in Yaphank. Two potential sites for identification stations would be Fishers Island and Riverhead. The Riverhead location could service the East End light traps.

Surveillance on Fire Island is complicated by travel issues and special treatment restrictions imposed under NPS rules. Generally, however, it is thought that surveillance there will follow the same general precepts as elsewhere in the County. Specific locations and procedures will be discussed in a separate FINS management document. It is most probable that the existing Fire Island network of CDC traps (see below, Section 3.5) will be expanded by SCVC adding New Jersey light traps in Saltaire, Davis Park and Fire Island Pines. Information generated from the SCVC trap network will be used, in conjunction with received FINS data, to determine the need to conduct mosquito management in the residential communities. The scope of vector control in FINS will be determined through a FINS-specific plan developed by the County and NPS.

CDC light traps are also good tools for testing the efficacy of adulticide applications. They can be optimally set within a proposed treatment area, and, if the application was for disease control purposes, the post-application sample can also be tested to determine if viral activity was curtailed. SCVC proposes to use CDC traps to routinely test the efficacy of adulticide applications. A trap should be set out the night prior to the application. Trap contents will be analyzed during the morning hours of the scheduled application to determine if the conditions that suggested the need for adulticiding have been maintained. Thus, this surveillance may lead

to reductions in the amount of pesticides used and result in a more focused use of resources. Traps should also be set post-application to determine the degree of population reduction caused by the treatment. Fluctuating weather conditions and other logistical considerations mean it will not always be possible to make a valid comparison with pre-application numbers. For this reason, control locations should be identified so as to provide means of appropriately interpreting the trap data.

Each year salt marshes in certain locations are plagued with mosquito infestations prompting many biting complaints from residents. Specific locations where these kinds of infestations routinely occur include:

- Bellport Village
- Brookhaven hamlet
- East Patchogue
- Mastic-Shirley
- Oak Beach
- Oakdale

Because these locations have historically resulted in repeated incidents of complaints regarding salt marsh mosquito broods, formal landing rate sites should be created in these areas. This type of surveillance is conducted by having personnel walk into and out of a grass field or marsh along the same path, wearing unprotected clothing on the lower half of the body (DEET may be worn on the upper part of the body). Depending on conditions, netting may or may not be worn about the head. The number of mosquitoes that land on the unprotected clothing on the lower part of the body during a several minute period is counted (mosquitoes do not bite through the clothing if appropriately thick pants are worn; because salt marsh mosquitoes that have disturbed from grass initially land low on the disturber, the DEET worn higher on the body does not appear to deter landing lower on the body), and then averaged to report the number of landing mosquitoes in each minute. The County intends to use a standard five minute exposure time.

This method is targeted at *Oc. sollicitans*, which is so aggressive and opportunistic that it leaves daytime resting places when disturbed and seeks a meal from the disturbing mammal.

Table 4. Landing rate and bite count data collection protocol

Wear solid colored clothing
Have all counters wear the same color clothing
Use no perfumes
Wear DEET only on upper body
Take counts from a standing position
Disturb area vegetation before beginning the counts
Count only mosquitoes that land within view
If work is conducted after sunset, use a red filter on any light source
Use a standard form to record information
Use whole numbers
Collect mosquitoes for identification with an aspirator, if needed
Use consistent time periods (five minutes is proposed)

As part of the overall program for assessing adult mosquito populations, SCVC will seek to establish trap stations for background (ambient) levels of mosquitoes. Reference sites can help understand the natural fluctuations in trap catches that occur even in the absence of control, due to weather conditions and natural mortality. These traps should be set where it is as certain as is possible that treatment will not occur. Fire Island traps already being monitored may serve the requirement to collect salt marsh data. A trap in an upland portion of the William Floyd Estate would also be a good placement. However, such an array would have a large geographic bias towards the Brookhaven Town south shore (which, admittedly, is a locus of County mosquito problems). The other decision that needs to be made is whether to use CDC or New Jersey light traps. New Jersey light traps fit the historical data base better, and so are preferred. However, the program is beginning to have a greater reliance on CDC light traps for management decisions. This means CDC traps would also suit program needs. Since CDC traps are generally managed by the ABDL while New Jersey traps are managed by SCVC, and these data will most likely be used to interpret variations in both CDC and New Jersey traps, and to provide management input for disease and vector control decisions, the decision may depend on manager perceptions of resource availability (both short-term when the decision is made, and long-term in terms of overall program resources). Such background stations will be established as resource availability allows, which is anticipated to require the completion of other pressing issues, such

as the construction and staffing of laboratory space and establishment of the progressive water management program.

3.4 Complaint Reporting and Hotline Response

Public complaints are a cornerstone of the County surveillance program (as is the case for most programs nationwide). SCVC responds to complaints regarding biting adult mosquitoes, larval breeding, clogged culverts, flooded marshes/swamps, and other sources of stagnant water. These complaints are received through the County's telephone complaint line. Over the past six years (2000 to 2005, the number of complaints have varied from 2,100 (2004) to more than 4,500 (2000), with a mean of 3,350. The calls are classified as to the general nature of the problem, and complaints regarding larval or adult mosquitoes comprise approximately 90 percent of the calls (see Table 5). All larval mosquito complaints receive immediate action. An inspector will visit the site within one to three days after receiving the complaint and submit a recommendation as to what action should be taken. Inspectors will provide information to homeowners, such as leaving door hangers listing information about the program and steps they can take on their own. As with other educational material, residents will be directed to the County's website for additional information. Inspectors will also determine the source of the problem. One outcome of the investigation may be to add the site to the mosquito breeding list. With adult mosquito complaints, isolated locations are responded to. If a large number of complaints from a relatively small geographic area are received, only a general response to the area will be made; not every complaint will be specifically responded to if it is clear that a brood of mosquitoes is the source of the problem. Each complaint location is mapped however, and these resident identifications of biting mosquito problems can help define areas where action may be needed. Public reporting has proven to be an excellent monitoring tool and will continue to receive an expeditious response.

Table 5. Service Requests, 2000-2005

	Service Requests	Adult Mosquitoes	Larval Mosquitoes
2000	4,590	1,784	2,806
2001	3,511	1,708	1,657
2002	2,608	1,343	1,069
2003	3,972	1,473	1,783
2004	2,154	673	927
2005	3,267	2,022	820
Mean percent		44.8%	45.1%

3.5 Disease Monitoring

Mosquito Sampling

Viral surveillance will continue to be conducted according to the latest CDC and NYSDOH guidelines and will likely continue to be primarily directed at EEE and WNV, with modifications to suit Suffolk County's unique environment. Suffolk County's ecological habitat diversity results in a large range of pests and vectors and also requires large amounts of traveling to implement surveillance and control measures. The large size of the County, coupled with resource limitations, has set some restrictions on where and how often traps can be placed or serviced. As an example, travel to the East End in summer is often impeded by resort traffic, meaning that efficiency is lost. Important in this regard is the current prohibition on taking County-owned vehicles home at night. If SCVC or ABDL personnel living on the East End could begin a day's work by collecting traps near home (and servicing them at night on the way home), instead of driving to Yaphank to pick up a suitable vehicle and then returning east, more traps could be set and serviced.

A major means of monitoring for virus activity is through CDC traps. CDC light traps collect host-seeking mosquitoes, and keep them alive. The mosquitoes are then preserved using cold storage to ensure any viruses present can be detected using molecular biological techniques or culturing. Currently, DNA analysis is used to identify WNV. Other viruses must be cultured and analyzed.

Trapped mosquitoes are identified and sorted by species in the laboratory. The groups of species ("pools") are then separated with the number of mosquitoes in each pool being noted. The ABDL has the technical means to test for WNV in mosquitoes at this time, but prefers to use state facilities for this purpose for worker safety reasons.

Culex mosquitoes that have had a blood meal and are seeking a location to oviposit are collected using CDC gravid traps (some other mosquitoes are also collected, but gravid traps generally attract a limited range of species). Gravid traps consist of a tub of stagnant, organic water with a collection net mounted over the tub. Gravid mosquitoes are attracted to the water and are drawn into the collection net by a fan. Gravid traps are adaptively placed in areas with a history of

WNV viral activity or the sampled presence of viral indicators, such as viral positive birds. The trapped mosquitoes are collected, sorted, kept cool, and tested, as are samples from CDC light traps.

The fresh water wetlands that are currently monitored by the County due to a history of viral activity are listed in Table 6 and Table 7. These wetland areas are monitored using CDC light and CDC gravid traps in fixed locations. EEE is strongly associated with red maple and white cedar swamps that are the habitat of the key mosquito species in the biology of EEE, *Cs melanura.* WNV can be found in a wide variety of habitats. Intense WNV activity has been detected in several fresh water wetlands throughout Suffolk County. Andrew Spielman (Harvard School of Public Health) has postulated that the amount of EEE in fresh water wetlands increases as the wetlands undergo successional changes as part of the natural maturation process, resulting in an increase in the amount of available habitat, for *Cs. melanura*, which breeds in underground crypts formed among tree roots.

Table 6. Fresh water wetlands with a history of EEE.

Wetland Location	**Recent detections**
Riverhead	1990,1994,1996
Robert Cushman County Park, Manorville	1994,1996
Bayview, Southold	1996
Camp Hero State Park, Montauk	1996, 2003
Shelter Island	1996
South Haven County Park	1996
Connetquot State Park	1997

Table 7. Fresh water wetlands with a history of WNV.

Wetland Location	**Year First Detected**
Belmont State Park	2000
Blydenburgh County Park	2000
Saltaire (Fire Island)	2000
Heckscher Park	2000
Canaan Lake	2000
Nesconset	2001
Watch Hill, Fire Island	2001
Smith Shores, Fire Island	2002
Meeting House Creek, Aquebogue	2002
William Floyd Estate	2003
Area adjacent to the County Jail, Riverhead	2004
Headwaters of the Carmans River, Yaphank	2004

With this in mind, SCDHS will revisit the County's fresh water wetlands that were last visited during an initial (1996) survey of potential EEE sites, as well as those that are not currently monitored, to determine if the ecology of these areas has matured sufficiently to support disease vectors by inventorying the types of vegetation present and looking for evidence of *Cs. melanura*. Examples of such areas are the major river corridors and the headwaters of the Peconic River. Sampling for *Cs. melanura* and other larvae should be performed and CDC light traps should be placed in the wetlands that have sufficiently matured to determine the population parameters of the mosquitoes currently inhabiting these areas. These areas should also be added to the list of fresh water wetlands that are currently monitored by the County on a regular basis. Extra field personnel and equipment, such as vehicles, would be necessary to sample these additional areas as well as more laboratory space for processing the samples generated as the result of increased surveillance.

Sampling frequency for these set locations is once a week, absent any indications of viral activity. If these are signs of local amplification, the frequency of sampling can be increased.

The County currently has 144 CDC traps of various kinds. 12 additional light traps will be received from the Long-Term Plan Early Action Project sampling efforts. The maximum number of trap set outs that can be managed with existing personnel is approximately 80 a week. The ABDL will try to increase the number of traps it can manage as personnel are added to staff. Additional sites for monitoring will be chosen based on history of viral activity or the presence of viral indicators, such as the finding of birds with WNV in the area. The best means of managing the light trap network is to establish "routes" that approximately cover a Township. Optimally, one person would be assigned to each of the smaller nine townships (Babylon, East Hampton, Huntington, Islip, Riverhead, Shelter Island, Smithtown, Southampton and Southold) and two to the much larger Town of Brookhaven. Traps are set out on an overtime basis, and because they must all be set out by dusk there is a limit to the number of traps that can be set out in any particular night. Weather and personnel management generally limit trap set outs to a maximum of two nights per person per week. This suggests that the maximal set outs that can be achieved with an optimally-sized staff would be on the order of 100 to 110. Expanding the trap network to something near to this size would increase the available information used to assess

the threat of potential disease transmission, including composition and abundance and seasonal and spatial distribution of mosquito vectors.

CDC trap contents must be processed in short order by the ABDL. Freezing trap contents leads to identification difficulties, as legs and other appendages are lost from certain specimens. Sample processing is a multi-step process. Biologists at the ABDL conduct a visual first sort of the trap contents, removing non-mosquito insects and making initial speciations of the trap contents. Entomologists then review the speciation under a microscope. Following the entomologists' reexamination of the samples, the biologists then create pools of 50 mosquitoes or less for shipment to NYSDOH, and complete all paperwork requirements. The entomologists are responsible for ensuring all packages meet ABDL and NYSDOH requirements.

Fishers Island represents a particular issue for the County. There is no direct public transportation from Long Island to Fishers Island. Travel must pass through Connecticut. The County currently conducts no viral sampling on Fishers Island, although it has appropriate EEE habitat, and WNV habitat.

An identification station would not address the need for viral surveillance. For viral surveillance, the preferred alternative is for the County to detail a technician whose major summer responsibility would be to collect samples from the traps on Fishers Island and return them to the ABDL each week. This person could utilize the ferry service that runs from Connecticut to Fishers Island, set a trap, stay overnight and return with the samples the following morning. This seems to be an extravagant expenditure of resources, however. An alternative (fiscally preferable from the County's perspective) would be to seek the services of a local pilot to fly from Fishers Island once per week to return samples, from May to October. This might be implementable as several of the residents own and pilot airplanes, and have expressed interest in supporting mosquito control efforts. The County currently details summer interns to Fishers Island to conduct larval surveillance and control (see below), and these personnel could be trained to collect and manage one or two CDC light traps.

FINS currently conducts its own viral surveillance. FINS has a network of CDC light and gravid traps (see Table 8). The Village of Saltaire currently operates a CDC light trap in Saltaire, primarily for disease surveillance reasons, because it has been a site of repeated viral detections

in mosquito pools and dead birds. Specifics regarding the future management of this Fire Island surveillance network will be spelled out in a Fire Island-specific plan.

Table 8. Location and trap types used in mosquito surveillance at FINS

Trap Type	Trap Location
CDC Gravid	William Floyd Estate East
	William Floyd Estate West
	William Floyd Estate Entrance
	Lighthouse
	Sunken Forest
	Watch Hill West
	Watch Hill
	Hospital Point
CDC Light	Lighthouse Tract
	William Floyd West
	Sailors Haven
	Watch Hill West
	Smith Shores

Pools of mosquitoes are currently sent to NYSDOH for viral analysis. The County can send samples every day, but results are generally not available for at least five days, due to time constraints that involve weekends, mailing, sample preparation, sample analysis, and data interpretation. Expansion of the ABDL to Biosafety Level-3 laboratory (BSL-3) (see below) would allow for local processing of mosquito samples, with overnight (or faster) results possible. This would increase the value of the information generated by the viral surveillance program immensely.

Arbovirus surveillance allows SCVC, in cooperation with SCDHS and NYSDOH, to gauge the potential for disease transmission and determine which control measure might be considered. SCDHS also remains in constant contact with NYSDOH to keep abreast of cases found elsewhere in the State as a gauge of possible threats faced here. SCDHS also maintains contacts with local veterinarians and stables for equine cases, and with hospitals for human cases of meningitis or encephalitis. This is a more informal surveillance process.

Avian Sampling

Through 2004, SCVC and SCDHS, in conjunction with NYSDOH and CDC, monitored for WNV using indicators such as unusual bird deaths or the number of dead birds sighted in an

area. The focal species were all corvids, such as crows and blue jays. Selections of dead birds were tested for actual presence of the virus. The presence of WNV-positive birds is a strong indicator of virus activity in an area and correlates well nationally with other virus measures.

SCDHS estimated that approximately 44 percent of birds selected for testing were actually WNV-positive in 2003. The number of dead birds found in an area actually does not provide information on the time or the place at which viral infection occurred, because of the mobility of crows. However, virus has been frequently found in mosquito populations at locations where a bird died of WNV. This is thought to be at least partially fostered by moribund birds infecting the local mosquito population.

The ABDL has developed the capacity to conduct these tests locally. Local bird tests can be confirmed with NYSDOH, since the local tests only require a buccal swab, and the bird corpse can then be forwarded onto Albany for culturing. The confirmations have shown that local testing detects approximately 93 percent of infected birds, and there has only been one false positive.

Recent observations in New Jersey suggest dead corvids as a surveillance tool may become obsolete in providing an early warning of virus activity, since fewer corvids may succumb to WNV infection than in the past due to immunity and/or decreased bird populations. In 2005, the local experience was that dead birds continued to be found, but no longer preceded positive detections in mosquitoes. The detection of positive birds in an area used to precede the detection of positive mosquitoes, primarily because any found dead bird had a relatively high probability of having died from infection. Mosquito pools are more random selections from the mosquito population, and so viral presence in mosquitoes needed to be much higher (relatively) for positive pools to be detected compared to the testing of dead birds. In 2005, apparently the greater resistance of crows to WNV meant that crows no longer served as an early sentinel for WNV presence.

Therefore, the County needs to consider developing some other forms of surveillance to detect the virus. This would help to avoid random sampling of the County, because, unlike EEE, WNV does not magnify in well-defined habitats. Nassau County does not face a similar problem, as its relatively compact size has allowed for a manageable grid of mosquito sampling sites that (in

essence) carpet that County. For Suffolk County, given that some traps need to be dedicated to sampling for EEE, even the current maximum set out potential of circa 80 traps does not allow for good sampling density to cover the entire County. Suffolk County needs a means of focusing its viral sampling efforts, therefore.

Other non-migratory bird species, such as house sparrows, may be useful as indicators of viral presence. Fledging sparrow deaths may serve as indicators of the presence of WNV in an area since they have been shown to be carriers of the virus. Unfortunately, as of yet, there has not been much research published on loss of young birds from the disease.

Viral activity in avian populations can also be monitored by:

- Netting
- Sentinel chicken flocks
- Obtaining blood samples from nestlings

Netting Technique

The capturing and handling of wild birds is controlled by federal law (Federal Migratory Treaty Act). Permits must be obtained from the US Fish and Wildlife Service (USFWS) and NYSDEC. Wild birds are often captured with a Japanese mist net. These fine black nylon nets are arranged in four shelves and come in various sizes. Small samples of blood sera (the fluid portion of the blood that contains antibodies) are removed from birds and the birds are banded before being released unharmed. Banding allows the agency to collect information on particular birds that can often be extremely useful for virus surveillance. For example, a bird sampled once and found to be negative, but recaptured and found to be positive, has obviously acquired the virus in the intervening time. Knowledge of the lifestyle of particular birds can mean that where the bird acquired the virus can be extrapolated, as well.

Site selection for net placement is based on the known or presumed presence of flyways. In Suffolk County, nets could be set up to cover the areas between CDC light and CDC gravid traps. The nets are set up before sunrise, and taken down by late morning. Nets are checked

every 15 minutes or less, and collapsed if it rains as hypothermia would be a concern in birds captured in the rain. Captured birds are held in opaque cotton bags until processed. Blood is drawn from the jugular or wing vein. The following characteristics are recorded:

- Species
- Band number
- Age
- Sex
- Weight
- Body fat
- Molting pattern

In Harris County, Texas, seven of nine species caught in suburban areas that tested positive for St. Louis encephalitis, are also found in Suffolk County. All seven (blue jay, Northern mockingbird, house sparrow, European starling, Northern cardinal, common grackle, mourning dove, and brown-headed cowbird) have been found to have positive antibodies for WNV, and so may be suitable for surveillance here. In Louisiana, testing found that the birds that had the highest percentage positive tests for WNV were the house sparrow and cardinal, and so Louisiana efforts focused on those species. Testing in Illinois also found that robins had a relatively high infection rate.

Sentinel Chicken Technique

Sentinel chicken serology is performed by placing chickens in an enclosed area for an extended period of time and testing their blood for the presence of antibodies to WNV and EEE. For example, the Los Angeles County West Vector Control District maintains and operates 15 flocks of chickens for this purpose, strategically placed throughout the District. The chickens are bled once every two weeks from May through October. Only a very small amount of blood is required from each chicken. ABDL staff similarly monitored sentinel chicken flocks in 2000.

However, none of the chickens was ever found to be seropositive, although some of the flocks were located in areas where crows and mosquito pools were positive. The County has therefore abandoned this effort. This lack of success may be because of the mosquito species that are potentially transmitting the virus to the birds were not entering the cages or could not successfully feed on the chickens. New Jersey programs had similar experience with sentinel chickens. Sentinel chicken programs in California monitor mosquito species that readily feed on caged chickens, such as *Culex tarsalis*. *Culex nigripalpus* is the dominant WNV vector in Florida, and also readily feeds on chickens. *Cx. pipiens*, is the northeastern WNV vector, and has difficulty feeding on birds that exhibit defensive behavior, as chickens often do when faced with feeding mosquitoes.

Blood Sampling

Obtaining blood samples from nestlings is another way in which viral activity in avian populations might be monitored. This method is most useful when early in the season, as the birds are sessile, and there is no potential of being seropositive from earlier exposure, or exposure elsewhere. A positive result would indicate that virus is circulating in that immediate area. However, nestlings often have left the nest just as WNV becomes of greatest concern (early August), which would mean changing surveillance tools at a key moment. In addition, permit issues make this kind of surveillance very difficult to administer.

It is clear that all of these techniques have disadvantages compared to the hitherto excellent dead crow surveillance system. Nonetheless, the County should seek to develop some new means of conducting sentinel surveillance for WNV. Whatever method is selected, testing of these samples could continue to occur in-house, with some samples sent to NYSDOH in Albany for confirmation and more general viral scans.

Laboratory Testing

In 2004, the ABDL acquired a Rapid Analyte Measurement Platform (RAMP). This piece of equipment detects WNV infection in dead birds by analyzing the viral RNA antigens present in an oral sample obtained by swabbing the inside of a bird's mouth, and can provide results within 24 hours.

RAMP is not used by SCDHS for mosquito testing because the technique it employs is not as sensitive as the technique used by Taqman (another system used to test genetic material for virus). Thus, SCDHS would run the risk of failing to detect the presence of virus if it relied on RAMP to process mosquito samples. The ABDL has compared the accuracy of RAMP to that of the Taqman, by analyzing samples obtained from 122 birds, including 28 American crows (*Corvus brachyrhynchos*) and 63 blue jays (*Cyanocitta cristata*), and found the accuracy of RAMP to be greater than 93 percent. Thus, RAMP seems to be a reasonably reliable tool for use on birds in WNV surveillance.

Historically, SCDHS has had a no-cost service with the NYSDOH laboratory to process mosquitoes for viral testing. Under this service, a limited number of mosquito samples (45 per week) were batched and sent to Albany weekly, which yielded results in approximately 10 to 14 days. In order to decrease the time needed to obtain testing results, in 2005 the County contracted with the NYSDOH laboratory. The ABDL can now choose to pay a nominal fee per sample ($25 per sample) and then send unlimited samples and unlimited shipments per week, which provides far superior viral surveillance than under the no-cost service. This contract has increased the number of mosquito samples tested and reduced the turnaround time for testing results to approximately five to six days, which is extremely valuable for formulating and directing mosquito control strategies.

With the proper laboratory facilities, the ABDL could employ the ABI 7900 HT (Taqman), which is a laser-coupled spectrophotometer, to perform a rapid version of the Polymerase Chain Reaction (PCR) test for viral testing. This machine uses the enzyme Taq polymerase ("Taq" is the bacterium *Thermus aquacticus*) and a fluorescent dye to detect WNV in mosquitoes. When optimized, results would be available in approximately two days, which further reduces the time needed for obtaining testing results from NYSDOH. Thus, local testing of mosquitoes by the ABDL would greatly reduce this turnaround time, but the principal factors preventing local testing are lack of staffing and lack of a BSL-3 facility.

Taqman and RAMP are specialized for WNV testing, but the County has a need to test for EEE, since it has often been detected here. Therefore, the County would like to conduct general viral surveillance to ensure that other arboviruses do not become established in the local mosquito

population without detection. This requires the use of virus culturing and standard PCR. The laboratory has the capability to perform standard PCR, but culturing and processing viruses also require that laboratory be equipped and certified at BSL-3, and meet certain Homeland Security requirements. The Long-Term Plan envisions, as part of an already planned laboratory upgrade, that the ABDL will be improved and certified to BSL-3 standards. Until the laboratory has arboviral testing capabilities, the ABDL will attempt to improve the efficacy of sample processing and the speed with which results are obtained.

3.6 Mosquito Surveillance and Control Unit Upgrades

A unit within SCVC is the Mosquito Surveillance and Control Unit. This section should be asked to perform additional tasks under the Long-Term Plan. In addition to the unit in general having expanded, typical surveillance duties, a subunit should be formed that has special responsibilities. This work unit has been informally designated as the "QA/QC" team.

Major tasks for the QA/QC team would include:

- special surveillance responsibilities. The QA/QC team should be responsible for conducting early spring sampling for *Cs. melanura,* and seasonal sampling for *Cq. perturbans* and of tire stockpiles. These require special sampling techniques, which the team should master and employ.

- larvicide effectiveness measurements. This is described in detail in Section 6.

- adulticide need testing. On the night before an adulticide application for vector control purposes, CDC light traps should be set to ensure that a need for the treatment can be demonstrated. Need for control should be based on a trap count of approximately 100 human-biting mosquitoes, although this number is not intended to be a threshold, per se. Section 7 discusses treatment triggers in more detail.

- In association with adulticide need testing, treatment efficacy measures should be made for each adulticide application (this is discussed in more detail in Section 7).

In addition, the QA/QC team could be assigned "research and demonstration" tasks, as needed, to test alternative technologies and methods that SCVC is considering adopting. An immediate use of this unit would be to develop an alternative bird sampling methodology, in conjunction with ABDL personnel, to keep WNV surveillance robust. Note that the QA/QC team does not make treatment decisions, but rather supplies information to support treatment decision-making processes.

3.7 Data Management

Surveillance

Monitoring of larval mosquitoes is conducted on a weekly basis usually starting on Mondays from late-April through mid-September in approximately 2,000 identified natural breeding areas. Parameters, such as the weather and water condition, that are associated with each inspected larval breeding area, will be recorded on paper forms and directly entered into hand-held GPS units. The forms are returned to the office each day, and information from the hand-held units is downloaded into the Vector Control Management System (VCMS) software database.

VCMS is a program by Advanced Computer Resources Corporation that offers a database, Geographic Information System (GIS), and a mobile data collection system for vector control agencies. The software logs requests for service, breeding data, pesticide application data, regulatory requirements, trap data, weather data, and other information collected or used by vector control agencies. The software aids SCVC in evaluating treatment efficacy and determining where future surveillance efforts should be concentrated. It has been suggested that the County investigate replacing these useful devices and system because it is difficult to interface the VCMS information directly into a standard GIS system. The loss of specificity may result in some data entry and system inconvenience; and VCMS has provided good technical support that is unlikely to continue absent a vendor-sponsored system. However, the utility of direct entry of data into a GIS system should reap great rewards in data management, and eventual conversion of data into information useful for management decisions.

Computer terminals placed at individual stations throughout the laboratory will be used to enter data resulting from processing samples obtained from surveillance activities. The use of

individual stations will streamline data entry by placing the terminal in the same area in which the sample is processed, preventing delays previously caused by sharing computers. These terminals will be linked to the County's GIS system in order to make the data accessible to all SCVC and SCDHS personnel as soon as possible.

Complaint Reporting and Hotline Response

All service request and response information will continue to be entered into hand-held GPS units in the field for download into the main system at a later time. This shall provide SCVC leadership with an accurate picture of current field conditions and activities, while eliminating the need for data entry back at the office and reducing the need for temporary staffing. The database will allow the County to monitor recurrences in the same area(s) weekly, monthly and yearly and determine the efficacy of any action(s) taken. Use of this database will enable the SCVC to rapidly identify and target problem areas, allowing resources to be more efficiently applied. In addition, the completeness of pesticide reports can be continually checked in this system, ensuring compliance with State reporting laws.

Data Analysis and Reporting

The Superintendent and the Director of the ABDL currently analyze collected data, with assistance from an entomologist, a GIS specialist, and ABDL staff. The type of data collected and resource allocation limit the scope of statistical analysis currently performed on collected data.

At this time, the ABDL Director produces a summary of the season's findings and annual work plans summarize operations from the previous year. However, a comprehensive annual report, including the season's results for all program areas, is not produced due to resource allocation limitations. This should change. It is clear that vector control programs need to justify their activities by collecting appropriate information and then making the information available to the public. A more in depth statistical analysis of laboratory and field data should be performed and an annual report should be produced detailing these results. This report could be posted on the County's website.

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4. Source Reduction

4.1 Background

Source reduction is also known as physical or permanent control, or source control. It consists of the elimination of larval habitats or the rendering of such habitats unsuitable for larval development. This can be accomplished by properly discarding old containers that hold water, or by more complex measures such as implementing progressive water management techniques, such as those generally known as Open Marsh Water Management (OMWM). The County believes that these kinds of progressive water management techniques control salt marsh mosquitoes and restore degraded habitat as an added benefit, as has been demonstrated in surrounding jurisdictions which use these processes. The Wetlands Management Plan discusses water management issues, and has been issued as a separate document (although it should be understood to be an appendix to the Long-Term Plan).

Mosquitoes require stagnant water to breed. Stagnant water is not necessarily "polluted," which is a term used by mosquito control professionals when describing water that has high organic matter content. In Suffolk County, most of the mosquito species that bite humans actually need water that is clear to relatively clear. Other species, however, do prefer or even need to breed in water that has a heavy organic burden.

The scope of mosquito breeding in Suffolk County includes at least 2,000 natural breeding areas and 100,000 artificial sites such as roadside catch basins, recharge basins, etc. Not included in this number are the innumerable domestic breeding sites that are created by property owners or their tenants.

The female adult mosquito will lay her eggs in practically any wet location. Breeding sites are often classified as permanent, transient, and containers. Some specific locations used by mosquitoes include:

- Ponds
- Puddles

- Tire Ruts
- Swamps
- Marshes
- Tree stumps
- Abandoned swimming pools
- Buckets
- Cans
- Bird baths
- Dirty gutters
- Stored tires

This list emphasizes locations over which people can take responsibility, although any place where water collects, even temporarily, is capable of supporting mosquito breeding. The IPM approach to mosquito control concentrates on eliminating mosquitoes before they become adults. This is because larval mosquito breeding sites can be readily identified and, generally, are relatively small in area. Once identified, most of these listed sites can be addressed to stop or minimize breeding. By contrast, the adult mosquito can fly many miles and cause problems over a much wider area. Thus, larvae are condensed within delineated habitats, but adults disperse widely following emergence.

The by-products of the activities of man have been a major contributor to the creation of mosquito breeding habitats. An item as small as a bottle cap or as large as the foundation of a demolished building can serve as a mosquito breeding area. Sanitation is a major part of all IPM programs, exemplified by tire removal, clearing waterways, catch basin cleaning, and container removal.

Public education is an important component of source reduction. Many agencies, including SCDHS, have public school education programs that teach children what they and their families can do to prevent mosquito proliferation. Suffolk County does more, including website maintenance, distribution of pamphlets, telephone hotlines, site visits, media relations and press releases, and presentations to citizens' groups and associations. Section 2 describes the proposed outreach program in some detail. Even minor housekeeping improvements can have significant disease risk reductions, because WNV vectors such as *Cx. pipiens* and *Oc. japonicus* often breed in artificial containers that hold water in and around the home. In fact, educating the public to eliminate or empty these is the only practical way to reduce these mosquito sources.

Source reduction can be the most effective and economical method of providing long-term mosquito control. It can help to reduce the need for pesticide use in and adjacent to the affected habitat. For example, the removal of discarded tires from the environment, whether they be individually littered items or tires that have been collected into a large stockpile, is widely noted as a basic step in reducing human health risks. This is because many encephalitis-bearing mosquitoes will use the temporary breeding habitats that invariably occur in tires. Tire removal from isolated dumpsites is also credited with aesthetic improvements, and tire removal reduces fire threats when the larger stockpiles are eliminated.

This portion of the Long-Term Plan will focus on household and institutional means of conducting source reduction. Water management will only be addressed briefly because of the extensive discussion contained in the attached Wetlands Management Plan.

4.2 Household and Institutional Source Reduction

Public education is the first step in realizing household source reduction. SCDHS has greatly expanded its role in educating the public about the public health importance of mosquito controls, and its educational outreach has been discussed above in Section 2. It is important to realize that many simple, common-sense steps such as maintaining items such as bird baths, or emptying containers in the yard, can have substantial impacts on risks associated with disease transmission. SCDHS will address these issues through presentations to groups and schools, its "Fight the Bite" and "Dump the Water" programs, and using the Citizens Advisory Committee newspaper insert, "Mosquito Control and Prevention at Home."

Tire disposal needs to be addressed. One way is by expanding the education program, as discussed in Section 2. It is important that other departments in County government (such as Parks and Public Works) understand the public health importance of removing littered tires when encountered. Similarly, since the towns are the level of government responsible for zoning and waste management in the County, they must understand the potential impact to public health when tires are not promptly removed from improper disposal sites. It may be that the County can provide useful resources to allow towns to address tire stockpile issues because of the public health.

Similarly, farmers, farm educators and advocates, and others involved in agricultural water use issues need to understand that the over-irrigation of fields can not only waste water, but generate mosquito breeding problems. This tends to be a greater problem in drier areas, and areas where soils are not as porous as they tend to be here. Thus, California is a leader in this source reduction field. Nonetheless, it may be that this may have greater resonance on the East End where water quantity and problem mosquito populations may become more pressing issues with greater population densities. SCVC already maintains certain water management structures in agricultural areas to drain standing water, and should expand its outreach efforts, perhaps through Cornell Cooperative Extension, to reach farmers on this issue, again, as discussed in Section 2.

Another addition to the program, discussed in Section 2, is storm water management structure maintenance. Not only should municipal (including County and State) departments be targeted, but those responsible for commercial properties and private homeowner associations should be engaged. Benefits associated with decreased flooding are most probably well-understood, but public health improvements are possible.

A survey of catch basins conducted as part of the development of the Long-Term Plan found that older systems tend to support more breeding than do newer systems. Since the technologies employed have not changed, the implication is that a lack of maintenance has impaired the functioning of these systems. This not only reduces compliance with local implementation plans for USEPA Phase II regulations, but threatens public health. As mentioned above in Section 2, these underground facilities are prime *Cx. pipiens* habitat, potentially the major vector for WNV.

Eliminating habitat for this mosquito species has immediate implications for the risks faced by people in the immediate vicinity of the water management structures receiving the maintenance.

Such maintenance is usually not very taxing; specialized equipment is used to remove standing water and accumulated fines, sands, leaves, and other debris from the basin. The removal of these materials will allow the hydraulics of the system to perform as designed, and also allow for more prompt drainage for those structures designed to infiltrate storm water to the aquifer system. Recharge basins are similarly maintained, although heavy equipment (such as bulldozers or backhoes) can be used to remove the accumulated material that may be impeding drainage. Many public works departments also remove vegetation from the basin as a maintenance activity; that may reduce mosquito breeding as well, as *Cq. perturbans* preferentially breeds in emergent vegetation, and other larvae may use vegetation for cover. In some systems, the basin vegetation may be a locally important ecotone, however.

Certain recharge basins are designed to maintain water as "ecological basins." These basins are intended to serve the local area as a surface water body. They should be maintained per design parameters (some have overflow devices, some have infill devices, some require vegetation maintenance, etc.). If these basins are supporting mosquito breeding, they should be managed as a natural water body, and environmental values fostered in these systems need to be considered prior to any treatment.

Implementation of certain storm water control devices holds the potential to create new mosquito breeding areas. These include those that are designed to remove pollutants from stormwater prior to discharge. Such devices, on Long Island, will mostly be used in the near vicinity of surface waters where high water tables are found, as otherwise it is standard practice to recharge stormwater back to the groundwater system. Nearly all manufacturers are aware of the public health concerns associated with mosquitoes due to WNV, and so have incorporated design features that minimize opportunities for within-structure breeding. Nonetheless, SCVC should have an opportunity to review any new designs proposed for use by the County, and should make outreach efforts to Towns and villages to ensure unsuitable devices are not being implemented locally.

The initiation of action by SCVC in household situations is often a complaint phone call. SCVC receives on the order of 3,000 phone calls for service per year. These are logged into the SCVC computer system, assigned to an inspection team on the basis of the geographical location of the complaint. Each complaint that is received, except when many adult mosquito complaints are from the same general area, is individually responded to within one to three days.

The initial response is to go to the complainant's house. State law and local implementing regulations allow SCVC wide latitude with regard to investigating and reacting to mosquito problems, so even if the complainant is not home some investigation will be undertaken.

CDC notes that sanitation is "a major part of all integrated vector management programs." Problems of neglect, oversight, or lack of information on the part of property owners are the types of problems most often faced by agency inspectors, nationwide. This is also true in Suffolk County.

In all cases, an immediate assessment of the problem is made. The issues to be addressed include:

- Determination of mosquito presence
- If mosquitoes are present, identification of the involved species
- Locating the source of the problem.

The primary investigative tool is larval dipping in potential source area water. Samples of larvae are returned to the laboratory for complete evaluation of the problem; however, field crews are trained in larval identification, as well. The larval stages and, very often, species involved, can be determined in the field. This can allow for accurate and effective choices should larviciding be determined to be the appropriate course of action. The follow-up laboratory identifications ensures that novel or unusual species are identified and noted, and as QA/QC for the field identifications.

Most often, the source of the problem is immediately obvious:

- a breeding habitat, such as an abandoned or poorly maintained swimming pool

- a recharge basin that retains water
- other sources of permanent water.

Permanent water mosquitoes often breed continuously, so that the water represents a constant source of new adults. Removing the water will break the breeding cycle, so that the numbers of adults will decline with the inevitable mortality of the adult mosquitoes. Therefore, draining the water source is the best solution for a local household mosquito problem.

Sometimes that is not possible, as when the source of water is as large as a swimming pool or relatively unmanageable as a recharge basin. Ecologically isolated, artificial bodies of water such recharge basins can be treated by stocking *Gambusia* (mosquito fish), or, preferentially, fish that do not have the potential for as great an environmental impact. If the water quality is marginally acceptable, fish will consume larvae even when there is a great deal of vegetative cover. SCDHS, through the ABDL, purchases these fish from commercial suppliers. This decision should be carefully considered, however. *Gambusia* are not native to Suffolk County, and therefore are a potential invasive species should they escape the basin. Secondly, the basin must retain sufficient water of high enough quality through the season for the *Gambusia* to survive. Third, if there are no native fish in the recharge basin, and it supports water seasonally, it may be functioning ecologically as a vernal pool, and serving as a home to a host of aquatic breeding insects and animals, especially amphibians, that are likely to be vulnerable to fish predation. For this reason, *Gambusia* has lost much of the luster that it once had for County source reduction purposes. Instead, the County should consider using species that are already found in County fresh waters for these purposes. One species that is especially promising is the fathead minnow (*Pimephales promelas*). Although this fish does not have the reputation for larval consumption that earned *Gambusia* its common name, it can and does prey on mosquito larvae. It is a very hardy fresh water fish, prefers slow-moving or still waters as habitat, tolerates high temperatures, high nutrient content water, relatively low dissolved oxygen conditions, and can live under a range of pH levels. Although not native to Long Island, it is established in many waterways throughout the County, apparently with little to no ecological impact. It is not as readily available from commercial fish hatcheries as *Gambusia* is, but New Jersey has raised them in hatcheries (so it is possible to stock them).

Still, due to potential impacts to areas where fish may not be found (or are not common), it is best if fish only be stocked in basins where they have been stocked before, and only after reconnaissance that shows there is no hydraulic exit from the basin (such as an overflow outlet) that could result in a release to ponds that may serve as fish-free environments. Potential new sites need to be carefully assessed in terms of the three major risk factors discussed immediately above. The introduction of any predator, although especially those that are non-native predators (such as tadpole shrimp or copepods), can have great, unintentional environmental impacts, and should only be undertaken at new sites if a careful impact assessment has been made.

Another attractive solution to mosquito breeding when recharge basins are slow to drain is to contact the basin owner and arrange for maintenance of the basin. Typically, this involves scraping and removing low permeability material that has settled from the retained stormwater, and created a more impermeable bottom than the basin was designed to have. Suffolk County basins typically are constructed deep enough not only to hold expected stormwater volumes, but to access high permeability sands if they are not immediately available. The ecology of the basin needs to be assessed prior to undertaking this step, however (if flooding is not an immediate, overriding concern). Recharge basins that retain water can be important water bodies, especially on Long Island where high soil permeability means that surface water is not plentiful in many areas. Those that permanently hold water can support a coastal plain pond type of ecology, fostering a great diversity of plants and animals due to fluctuating water levels. Recharge basins that only intermittently hold water may serve as vernal pools, and be key habitat for biota such as amphibians that need the isolation from many predators that impermanent water bodies provide.

This assumes that the basin is not an anoxic, eutrophied, contaminated pool of water that is not utilized by much other than breeding by *Cx. pipiens*. Many of these poorly draining basins do not provide the necessary water storage and recharge functions they were designed for, as well, and so maintenance would be required even absent a mosquito problem.

A stop-gap measure for such systems, until maintenance can be arranged for, would be to apply larvicides to control breeding. Timed release formulations of larvicides such as *Bacillus thuringiensis var israelensis* (Bti), *Bacillus sphaericus* (Bs), or methoprene can be in order. Larvicides can be applied in other kinds of enclosed water bodies, such as the ecologically-sound

basins discussed above, as the research and risk assessment has generally found little to no evidence of ecological injury from these biorational compounds. The discussion of larvicide use will be more complete, below.

For purely artificial, non-ecological systems such as an abandoned cistern or swimming pool, larvicide applications are an effective means of breaking the breeding cycle. Where more complex, natural systems seem to be the source of the homeowner complaint, such as a fresh or salt water wetland, then more sophisticated intervention is required.

Once an inspection team has investigated a site, it will discuss its findings and actions with the homeowner, with the intention of teaching the homeowner, should the cause of the problem be self-inflicted, or the neighbor (or municipality or agency), should the source be nearby and identifiable. Pre-printed check-off cards are used when the involved landowners are not at home. These cards invite follow-up phone calls to explain the findings and actions taken, and to try to ensure that the problem does not reoccur by conducting appropriate homeowner education.

Rarely, and only with extensive, although potentially time-compressed investigation, would adulticiding be considered in response to homeowner complaints. A nexus of complaints can be an important surveillance tool. For example, some mosquitoes, such as the tree hole (and tire) breeding mosquito *Oc. japonicus*, can be difficult to capture in the most common surveillance traps. Complaints spurring observations of these mosquitoes can result in determinations that control of the adults is or is not necessary.

Although the Long-Term Plan intends to increase the long-term, set surveillance network, not all salt marsh areas in the County will be adequately covered by the New Jersey or CDC light traps. It is possible that a brood will be generated in one or more marshes, and cause a localized, but intense problem that may require adult control, without causing any increases in surveillance counts. For areas such as Fire Island, where geographic conditions make regular surveillance difficult, historically resident complaints have served as a valuable means of determining when more precise surveillance needs to be initiated. Adulticiding threshold and conditions are discussed in much more detail below; it needs to be understood that several complaint calls to the SCVC hotline are **not** the necessary and sufficient conditions to initiate an adulticide application in a neighborhood.

Each year during the off-season, prior to the development of the coming year's brochure, field personnel from SCVC should interact with the health educators from SCDHS. This will allow transfer of information from the field to the educators regarding the kinds of persistent problems that are not being reduced through current education programs. In addition, field crews will be made aware of the current focus and ranges of materials used by the educators, which should enhance the field crews' education efforts, as well.

It should to be noted that the County Administrative Code (Section A8-5) specifies that environmental improvements are one possible criterion to justify maintenance dredging. Public benefits must be demonstrated prior to allocation of County resources for maintenance dredging projects. Any future dredging proposal that cites vector control benefits as a public benefit will require separate review.

4.3 Water Management

The Wetlands Management Plan, together with its associated Appendix, the Best Management Practices manual, is a part of the Long-Term Plan, and is understood to be appended to the Long-Term Plan. The following, largely excerpted from the Executive Summary of the Wetlands Management Plan, summarizes this most important element of the Wetlands Management Plan.

Overview

Through this Wetlands Management Plan, Suffolk County plans to address the vector control needs for all 17,000 acres of tidal wetlands in Suffolk County. Within the universe of wetlands that require operational attention of Vector Control, wetlands health (maintenance and, where feasible, restoration of functions and values) will be a permanent goal. The approach of natural reversion, best management practices, and selective major marsh restoration will be a radical departure from the current program of maintenance of the legacy grid ditch water management system. This management of wetlands for mosquito control purposes will be undertaken within the larger context of a comprehensive, County-wide Integrated Marsh Management program.

It is estimated that approximately 4,000 acres of tidal wetlands will be left alone to undergo reversion, because of low mosquito breeding potential and/or distance from points of dense populations of people. In those areas, natural processes will gradually undo the construction of

ditches across the marshes. In the long run, reversion is not necessarily ecologically optimal; thus, other restoration options may need to be considered for purposes other than vector control as the County, through its Wetlands Stewardship Committee, develops a definition of marsh health and uses that to derive a comprehensive marsh management plan, creating an Integrated Marsh Management program.

Low-impact Best Management Practices and progressive water management will be considered for implementation at over 4,000 acres of tidal wetlands that have been identified as mosquito breeding problem areas. These 46 separate locations are the sites that currently receive aerial larvicide treatments. The goals of this initiative are to reduce the amounts of larvicide applied in these marshes, and, according to the development of the Integrated Marsh Management program, achieve habitat enhancement and marsh restoration, including maintaining or increasing biodiversity and controlling *Phragmites*. Most of the projects that achieve these goals will undergo further environmental reviews, ranging from careful, thorough scrutiny due to the possibility of major changes to the wetlands associated with major projects to more modest evaluations building on the comprehensive reviews compiled in the DGEIS for the Long-Term Plan. These projects will be evaluated in light of overall natural resource goals, as outlined by various local specialists, regulators and interested parties, in the context of the Integrated Marsh Management program.

The remaining 9,000 acres of the County's salt marshes will undergo assessment by the County in cooperation with local government, regulators, and other interested parties over the coming years, with some subject to Best Management Practices or other marsh restoration efforts, and others subjected to reversion processes. The policy in these areas will be one of presumptive interim reversion (i.e., no ditch maintenance unless deemed necessary for ecological or mosquito control purposes). It is expected that less than four percent of the County's tidal wetlands (less than 600 acres) will be subject to ditch maintenance over the next decade.

Most identified mosquito problems in these areas will most likely be capable of being addressed using less intrusive techniques that have been identified as having fewer impacts on the existing hydrology of the marshes. However, overarching natural resource concerns and policies regarding the County's marshes may require more active management of certain sites to achieve

identified goals; in those cases, the mosquito management needs of a site may not be the driving force behind the scope of the restoration effort.

Ditch Maintenance Policy

Suffolk County has inherited a legacy of approximately 17,000 acres of tidal wetlands, wetlands which have been fundamentally altered from their natural state. In the 1920s and 1930s, these tidal wetlands were substantially grid ditched, in an effort to remove stagnant water and mosquito-breeding habitat. Natural features, such as ponds and pannes, were affected in many settings, and biological communities in the wetlands were altered.

The Wetlands Management Plan represents a significant departure from seven decades of grid ditch maintenance policy. Instead of committing to maintain the grid ditch network as a means of controlling mosquitoes, Suffolk County will instead apply more nuanced criteria to determine the best means of managing its salt marsh resources. For now, plans include a presumptive policy of reversion, where wetlands that pose no mosquito problems will remain untouched while long-term plans for restoration are developed and implemented. Existing water management systems (ditches, culverts, and other structures) will normally be either left alone, if not needed for mosquito control, or upgraded to Best Management Practices (BMPs) as outlined in the Wetlands Management Plan. In some cases, implementation of BMPs is not immediately feasible due to lack of pre-project information or institutional factors such as land manager policies. Implementation of BMPs may also not be immediately feasible due to lack of resources. For instance, if major tidal flow restoration is desirable but is currently too expensive because it involves major road work, interim measures should be taken while these resources are sought if the alternative is a loss of habitat and/or an increased reliance on pesticides. In addition, extensive project reviews may determine that implementation of BMPs is not warranted due to environmental considerations.

Assuming Long-Term Plan water management policies are implemented, the general presumption will be against maintenance of ditch systems. However, in limited circumstances, existing structures may be maintained when the following conditions are met:

- Deterioration of or damage to structures is resulting in a significant mosquito problem, as evidenced by larval and/or adult surveillance, serious enough to require control. An example would be a collapsed pipe that restricts tidal flow and results in a need to larvicide an area. Or:

- Failure to maintain the structures would result in the loss of resource values, such as fish passage or tidal flow, or loss of vegetation due to fresh water impoundment. Or:

- Failure to maintain the structures would result in a hazard or loss of property as a result of flooding.

Benefits to be expected from the work include:

- Maintaining or reconstructing the existing structures will improve water circulation or provide fish habitat sufficient to reduce the need for pesticide application.

- Maintaining the structures is compatible with habitat values that existed prior to the failure or deterioration of the structures.

- Maintaining the structure will prevent flooding or other hazards.

Constraints on any maintenance of a pre-existing ditch system include:

- The structures will be maintained essentially in-place and in-kind.

- Disruption of wildlife habitat due to construction will be minimized by limiting work areas and/or by using seasonal constraints.

- Listed species will not be adversely impacted.

- Interim maintenance will not lead to excessive drainage that would result in a loss of wetlands values.

- The action will not lead to increased or more direct conveyance of inputs from storm drains or other structures.

- The action will not preclude the implementation of BMPs when resources and/or institutional considerations allow.

Given the above, it is expected that less than 50 acres of tidal wetlands per year will be subject to ditch network maintenance. All maintenance will be summarized annually, and will be conducted in accordance with a Memorandum of Understanding between the SCDHS Office of Ecology and the Suffolk County Department of Environment and Energy (SCDEE). The development of the County-wide Integrated Marsh Management program may lead to significant changes in how interim actions are considered.

Progressive Water Management

The proposed strategy includes the goal of conducting a broad variety of best management practices and, specifically, to implement the kinds of progressive water management (practices that are sometimes labeled as Open Marsh Water Management, or OMWM). In many proximate jurisdictions, these kinds of marsh management actions are considered to be part of overall wetlands restoration programs, utilized as a means of achieving mosquito control and also enhancing certain natural resource processes associated with salt marshes. All mosquitoes spend larval stages as aquatic organisms, and source reduction is an essential component of mosquito control as practiced through IPM. Source reduction efforts through progressive water management lead to impressive reductions in successful mosquito breeding, and so lead to major reductions in the number of applications and overall usage of pesticides. In addition, this kind of water management also has the potential to increase overall marsh habitat diversity and wildlife values. This is because progressive water management is predicated on improving in-marsh water quality and overall fish habitat so that killifish have better, more consistent access to potential mosquito breeding areas. Killifish are voracious consumers of mosquito larvae, and so prevent mosquito breeding from being successful wherever they can access breeding locations. Commonly-identified by-products of improved killifish habitat can include:

- improved water fowl and wading bird habitat due to greater open water area
- decreased *Phragmites* vigor and extent because of increased in-marsh salinities

- greater ecological connections to the surrounding estuary, primarily because of improved nekton foraging supported by greater water circulation in the marsh system.

For the Wetlands Management Plan, all projects will consider mosquito management implications. However, the project scope and final design of all projects will be developed cooperatively by the County is conjunction with local governments, regulators, and other interested parties. Because of keen interest and many comments received on the original proposed means of evaluating projects, the County has expanded the review process. All proposed projects will require SCVC to consult with local natural resource managers, and to receive regulatory approvals from the New York State Department of Environmental Conservation (NYSDEC). A Wetlands Stewardship Committee has been established with a diverse membership that includes all groups of marsh management stakeholders. The Wetlands Stewardship Committee will be notified of all but the projects of no to little potential impact. It will review all projects with the potential for major impacts, and any other project that its membership deems to need such attention (see Figure 4). Suffolk County will not be able to participate in projects that do not receive Stewardship Committee approvals, and this Committee will make recommendations to the Council on Environmental Quality regarding the need for further environmental reviews. All projects requiring the use of management activities in BMP classes 5 to 15 will necessarily undergo some form of further environmental reviews. In addition, the Stewardship Committee has a priority to create a definition of marsh health, and to use that definition to create a comprehensive marsh management plan for the County, one that will be the basis of an Integrated Marsh Management program.

This holistic approach to the design and evaluation of potential mosquito control and ecological enhancement projects has been demonstrated for the first time on Long Island, as part of the Wetlands Management Plan, at the Wertheim National Wildlife Refuge. There, the land manager (USFWS) and the primary regulator (NYSDEC) worked together with the County and its consultants to ensure that natural resource manager concerns and vector control goals were all addressed. This project was also important as the State issued a permit to the County contingent on a new County commitment to conduct monitoring and provide documentation of the effects of the project, which generally had not been attained by earlier marsh management demonstration

projects. Continued cooperation between federal and state agencies will be critical to ensure that other progressive water management projects will be implemented throughout Suffolk County.

Wetlands Management Plan Approach

The Wetlands Management Plan consists of seven sections, the first of which addresses goals and numerous objectives. In the second section, a framework for selecting, designing, evaluating, and assessing projects is discussed. Key features include the creation of a Stewardship Committee to review and approve the major projects, and identification of the task to develop a marsh health definition, and use of that concept to create a comprehensive Integrated Marsh Management program that extends beyond mosquito control concerns. The Stewardship Committee is comprised of the following groups and organizations:

Estuary programs:
- LISS representative
- PEP representative
- SSER representative

State
- NYSDEC Region I
- NYSDEC Bureau of Marine Resources
- NYSDOS

County
- County Legislature
- County Executive
- SCDHS
- SCDPW
- SCDEE
- Suffolk County Department of Planning
- Suffolk County Department of Parks
- Council on Environmental Quality

Local
- Town representative (based on project location)
- Trustees representative (based on project location)

Non-governmental Organizations
- Two appointed by County Legislature
- Two appointed by County Executive

The Stewardship Committee will be informed of all projects requiring use of BMPs 3 to 15. It will automatically have project review of any project including BMPs 10 to 15, and all other

projects where members determine that review would be beneficial. Suffolk County will not be able to participate in reviewed projects that do not receive Stewardship Committee approval. The Stewardship Committee can also make recommendations to the Council on Environmental Quality regarding the environmental reviews required to fulfill SEQRA obligations.

The Stewardship Committee will also develop a definition of marsh health. The Long-Term Plan process (in the Literature Search, Book 9 Part 1) developed those presented below in Table 9.

Table 9 – Proposed first-order indices for marsh health in Suffolk County.

Health Indicator	**Good Condition**	**Alert Status**
Marsh stability	Net loss of vegetated wetland <1% per year	Net loss of vegetated wetland >3% per year
Plant health (for *S. alterniflora* only – health of the high marsh presumably threatened by *Phragmites* invasion rather than vegetation loss as in the low marsh)	<5% of vegetated marsh with stem densities below 100/m^2 or total below-ground biomass from 0-20 cm >3000 g/m^2	>10% of vegetated marsh with stem densities below 100/m^2 or total below-ground biomass from 0-20 cm <1500 g/m^2
Invasive species	<30% *Phragmites* sp.	>50% *Phragmites* sp.
Resident finfish	Killifish group represented in most or all suitable habitats	Killifish group absent from >30% of suitable habitats
Species of Interest (e.g., marsh sparrows, terrapins, forb plants, others)	Stable population or consistent use of marsh by species of special State or Federal status	No species of concern present or viable
Temporal trends	Selected indicator does not trend negatively in 3 or more consecutive years	Selected indicator trends negatively in 3 or more consecutive years
Note: marsh characteristics between Good and Alert condition should be considered to be Of Concern and monitored closely		

The definition of marsh health will be used to develop a comprehensive marsh management plan for the County. This marsh management plan will incorporate all aspects of marsh management needs, including vector control, tidal flow restoration, natural resource augmentation, other environmental improvements, aesthetics enhancement, and restoration of marshes back to

conditions that existed prior to earlier marsh manipulations. This plan will serve as the basis for an Integrated Marsh Management program, one that includes but extends beyond mosquito control concerns.

In section three, the 15 Best Management Practices and four Interim Management Actions are discussed (Tables 10-14). The actions are aimed at reducing mosquito populations utilizing methods that either minimizes potential environmental change, or maximizes the enhancement of particular natural resource values.

Section 4 and Section 5 of the Wetlands Management Plan address plan implementation and resource needs of SCVC to undertake this Wetlands Management Plan, respectively. The need for streamlined and dedicated State processes is highlighted. Vector control program needs may be eligible for restoration grant opportunities, as well as the Suffolk County Water Quality Protection and Restoration Program (the Quarter Percent Sales Tax). Section 6 establishes a Timeline for implementing the Wetlands Management Plan, and in Section 7 the County's salt marshes are prioritized in terms of those marshes where mosquito control needs are greatest, sites that appear to be best suited for reversion, and those areas requiring closer study before determining overall management needs. The intent of the first three years of projects is to establish a track record for the County, in terms of technical competency with these projects, but also to demonstrate a willingness to work with landowners and other interested parties to develop projects that meet with needs and desires of all concerned with a particular marsh through a cooperative process. It seems likely that until an overall County wetlands management strategy is developed by the Stewardship Committee, marsh restoration projects will be limited to Wertheim National Wildlife Refuge and County holdings.

Fresh Water Wetlands

In New York State, fresh water regulations do not allow for much manipulation of the existing hydrology of the marshes. This means that there are very few options in terms of mosquito-related water management and restoration. Source reduction (with an emphasis on manmade breeding sites) and larval control (with the use of biorational larvicides) are the main means of addressing mosquito problems associated with fresh water wetlands.

Underlying Data and Interagency Approach

This plan is based on a tremendous amount of collaboration among agencies within the Wetlands Subcommittee of the Technical Advisory Committee. It is also the result of an exhaustive literature review and comprehensive field work, which is reflected in Task 3 (Literature Review) and Task 7 (21 representative wetland areas, totaling over 2,000 acres, have been evaluated in detail). The first digital tidal wetlands map, for all County wetlands, has been produced, and the Remote Sensing project is expected to provide a continuing and cost-effective means to implement the long-term program.

Table 10. Management Activities with No or Minimal Impacts

BMP	Action	Factors to Consider	Potential Benefits	Possible Impacts	Equipment to be used	General Compatibility With Tidal Wetlands 6 NYCRR Part 661
BMP 1.	Natural processes (reversion/no action)	- Default option - Land owner prefers natural processes to proceed unimpeded - Natural reversion is actively infilling ditches - No existing mosquito problem	- Return to pre-ditch hydrology - More natural appearance/processes - Requires no physical alterations	- Possible increase in mosquito breeding habitat, creation of problem - Loss of ditch natural resource values - Loss of tidal circulation - Phragmites invasion if fresh water is retained on marsh - Drowning of vegetation if excess water is held on marsh	Not applicable	NPN
BMP 2.	Maintain/repair existing culverts	- Flooding issues - Are existing culverts adequate for purpose? - Are existing culverts functioning properly?	- Maintain existing fish and wildlife habitats - Maintain tidal flow and/or prevent flooding	- Continue runoff conveyance into water bodies - Roads & other associated structures	- Hand tools (minor maintenance) - Heavy equipment for repair	GCp

Please note that other jurisdictions besides NYSDEC may also regulate activities in wetlands.

Table 11. Management Activities with Minor Impacts

BMP	Action	Factors to Consider	Potential Benefits	Possible Impacts	Equipment to be used	General Compatibility With Tidal Wetlands 6 NYCRR Part 661
BMP 3.	Maintain/ reconstruct existing upland/ fresh water* ditches	- Flooding issues - Are existing ditches supporting flood control? - Are existing ditches needed for agricultural uses?	- Maintain existing fish and wildlife habitats and hydrology - Prevent or relieve flooding - Support turtle habitat - Provide fish habitat	- Continue runoff conveyance? - Perpetuate existing degraded conditions - Excess drainage	- Hand tools (minor maintenance) - Heavy equipment for reconstruction (rare)	NPN, GCp (6 NYCRR Part 663)
BMP 4	Selective Maintenance/ Reconstruction of Existing Salt Marsh Ditches	- Local government issues and concerns resolution - SCDHS Office of Ecology review - Mosquito breeding activity - Land owners long-term expectations - Overall marsh functionality - Ditch maintenance is to be selective and minimized	- Enhance fish habitat - Maintain existing vegetation patterns - Maintain existing natural resource values - Allow salt water access to prevent/control Phragmites - Reuse pesticide usage	- Perpetuate ongoing impacts from ditching (lack of habitat diversity)	- Hand tools (minor maintenance) - Heavy equipment for reconstruction	NPN, GCp

Please note that other jurisdictions besides NYSDEC may also regulate activities in wetlands.

Table 12. Management Activities with Potentially Significant Impacts

BMP	Action	Factors to Consider	Potential Benefits	Possible Impacts	Equipment to be used	General Compatibility With Tidal Wetlands 6 NYCRR Part 661
BMP 5.	Upgrade or install culverts, weirs, bridges	- Flooding - Flow restrictions - Associated marsh impacts - Cooperation from other involved departments	- Improve tidal exchange and inundation - Improve access by marine species - Increase salinity to favor native vegetation - Improve fish habitat & access	- Negative hydrological impacts - Changes in vegetation regime	- Heavy equipment required	GCp, P, PiP
BMP 6.	Naturalize existing ditches	- Grid ditches - Mosquito breeding activity - Landowner needs - In conjunction with other activities	- Increase habitat diversity - Increase biofiltration - Improve fish habitat and access by breaching berms	- Hydrology modification - Minor loss of vegetation - Possible excess drainage	- Hand tools (minor naturalization) - Heavy equipment for major	GCp
BMP 7.	Install shallow spur ditches	- Mosquito breeding activities - Standard water management not successful (continued larviciding)	- Increase habitat diversity - Allow higher fish populations - Improve fish access to breeding sites	- Drainage of ponds and pannes - Hydraulic modification - Structure not stable	- Preferably hand tools	GCp
BMP 8.	Back-blading and/or sidecasting material into depressions	- Mosquito breeding activities - Standard water management not successful (continued larviciding)	- Improve substrate for high marsh vegetation - Compensate for sea level rise or loss of sediment input - Eliminate mosquito breeding sites	- Excessive material could encourage Phragmites or shrubby vegetation - Materials eroded so that application was futile	- Heavy equipment required	Usually NPN or GCp; could be PiP or I
BMP 9.	Create small (500-1000sq. ft) fish reservoirs in mosquito breeding areas	- Mosquito breeding activities - In conjunction with other water management - Natural resource issues	- Increase wildlife habitat diversity/natural resource values - Improve fish habitat - Eliminate mosquito breeding sites - Generate material for back-blading	- Convert vegetated area to open water with different or lower values	-Heavy equipment required	PiP

Please note that other jurisdictions besides NYSDEC may also regulate activities in wetlands.

Table 13. Management Activities with Potential Major Impacts

BMP	Action	Factors to Consider	Potential Benefits	Possible Impacts	Equipment to be used	General Compatibility With Tidal Wetlands 6 NYCRR Part 661
BMP 10.	Break internal berms	- Water quality (poor) - Standing water (mosquito breeding) - Impacts on structural functions	- Allow access by marine species - Prevent waterlogging of soil and loss of high marsh vegetation - Improve fish access to mosquito breeding sites - Prevent stagnant water	- Changes in system hydrology - Excessive drainage of existing water bodies - Introduction of tidal water into areas not desired	- Hand tools (minor) - Heavy equipment (major)	Pip
BMP 11.	Install tidal channels	- Improve water quality - Tidal ranges and circulation - Increase salinity (invasive vegetation) - Natural resources enhancement	- Improve tidal exchange - Improve access by marine species - Increase salinity to favor native vegetation - Improve tidal inundation - Improve fish habitat	- Changes in system hydrology - Excessive drainage or flooding of uplands - Increase inputs from uplands into water body	- Heavy equipment	PiP
BMP 12.	Plug existing ditches	- Improve fish habitat - Tidal ranges and circulation - Prevent upland inputs - Natural resources enhancement	- Return to pre-ditch hydrology & vegetation - Reduce pollutant conveyance through marsh - Provide habitat for fish & wildlife using ditches - Retain water in ditch for fish habitat - Deny ovipositioning sites	- Changes in system hydrology - Reduce tidal exchange - Reduce fish diversity in ditches due to lack of access - Impoundment of freshwater could lead to freshening & Phragmites invasion - Possible drowning of marsh vegetation	- Heavy equipment	PiP or I
BMP 13.	Construct ponds greater than 1000 sq.ft.	- Landowner's needs - Water fowl habitat - Natural resources enhancement - Aesthetic improvements	- Increase habitat values for targeted species and associated wildlife - Improve habitat for fish - Eliminate mosquito breeding sites	- Changes in system hydrology - Convert vegetated areas to open water with different and possibly lower values	- Heavy equipment	PiP
BMP 14.	Fill existing ditches	- Landowner's needs - Aesthetic improvements - To restore pre-ditch hydrology - Vegetated areas	- Return to pre-ditch hydrology and vegetation - Reduced likelihood of pollutant conveyance through marsh - Create vegetated habitat to replace that lost by ditches or by other alterations - Deny mosquito breeding habitat by eliminating stagnant ditches	- Potential to create new breeding habitats if ditches are not properly filled or by making the marsh wetter - Loss of ditch habitat for fish, other marine species & wildlife using ditches - Loss of tidal circulation - Phragmites invasion if freshwater is retained on marsh - Drowning of vegetation if excessive water is held on marsh	- Heavy equipment	PiP or I
BMP 15.	Remove dredge spoils	- Increase wetland habitat	- Convert low-value upland to more valuable wetland habitats - Eliminate mosquito breeding sites	- Could result in new breeding sites if not carefully designed - Major change in local topography	- Heavy equipment	PiP

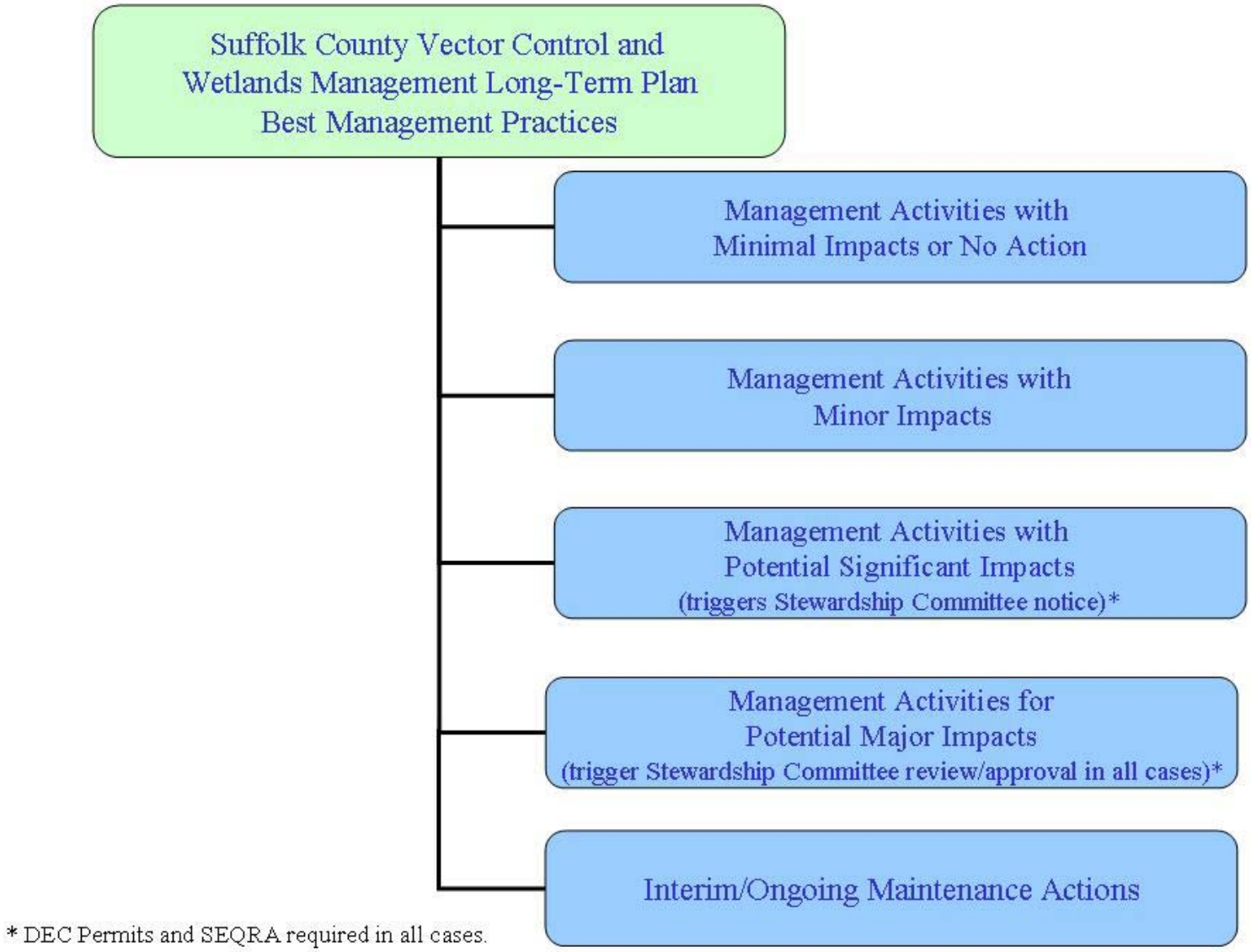
Please note that other jurisdictions besides NYSDEC may also regulate activities in wetlands.

Table 14. Interim Management/Ongoing Maintenance Actions

Interim Action	Action	Factors to Consider	Potential Benefits	Possible Impacts	Equipment to be used	General Compatibility with Tidal Wetlands 6 NYCRR Part 661
IMA 1.	Natural processes (No action reversion)	-Presumptive interim action	- Non-intervention in natural system	- Non-intervention in natural system	- Non-intervention in natural system	- Non-intervention in natural system
IMA 2.	Selective ditch maintenance (Standard Water Management)	- mosquito breeding activity - water quality (poor) - improve fish habitat	- Enhance fish habitat - Maintain existing vegetation pattern - Improve fish access to breeding sites - Increase fish and wildlife habitat diversity - Increase biofiltration - Improve fish habitat and access by breaching berms	- Perpetuate ongoing impacts from ditches - Hydrology modification - Minor loss of vegetation - Possible excess drainage of marsh surface	- Hand tools (Minor) - Heavy equipment (Major)	NPN, GCp
IMA 3.	Culvert repair/maintenance when tidal restrictions are apparent	- improve water quality - restore pre-restriction hydrology -mosquito breeding activities	- Maintain existing habitat - Maintain existing flows and/or prevent flooding	- Continue runoff conveyance into water bodies - Potentially inadequate water transmission	- Heavy equipment	GCp
IMA 4.	Stop-gap ditch plug maintenance	- prevent upland inputs - increase wetland habitat - sustain fish and wildlife habitat	- Return to pre-ditch hydrology & vegetation - Reduce pollutant conveyance through marsh - Provide habitat for fish & wildlife using ditches - Retain water in ditch for fish habitat - Deny ovipositioning sites	- Reduce tidal exchange - Reduce fish diversity in ditches due to lack of access - Impoundment of freshwater could lead to freshening & Phragmites invasion - Possible drowning of marsh vegetation - Impermanent approach (likely to fail within 5 years)	- Heavy equipment	GCp

Please note that other jurisdictions besides NYSDEC may also regulate activities in wetlands.

Figure 4. Overall Wetlands Management Hierarchy



Wetlands Management Regulatory Setting

The Federal government regulates wetlands under the Federal Water Pollution Control Act (1972) ("The Clean Water Act"), which covers all "waters of the United States" which may have been or are used in interstate or foreign commerce. Wetlands are defined in accordance with three criteria:

- Hydrology
- Vegetation
- Soils

Wetlands are defined as "waters of the United States." This definition has received some modification through a 2001 Supreme Court decision (*Solid Waste Agency of Northern Cook County v. United States Army Corps of Engineers*), which limits the Federal government in its regulation of isolated wetlands that are not hydraulically connected to other waters. The discharge of dredge or fill material or any kind of construction in a wetland requires a permit from the US Army Corps of Engineers, under the Clean Water Act (§ 404). The Federal government does not regulate "adjacent" areas near wetlands.

The Federal government can also regulate wetlands under Section 10 of the Rivers and Harbors Act of 1899 (33 USC 403). Section 10 regulates navigable waters and includes activities such as beach nourishment, dredging, filling, and the construction of boat ramps, piers, pilings, and shore protection.

In addition, President Bush established a federal policy of "no-net loss" of wetlands, on Earth Day, 2004.

The National Marine Fisheries Service has review responsibilities for actions involving activities seaward of the high tide line, and special responsibilities for designated Essential Fish Habitats, which may affect wetlands projects.

The New York State Department of State (NYSDOS), through the Coastal Zone Management Act (1972), was delegated authority to address coastal zone problems, including environmental issues, which can include wetlands (see below).

There are two Federal estuary programs that potentially affect the County's wetlands, the Long Island Sound Study (LISS) and the Peconic Estuary Program (PEP). Both are administered by USEPA.

The LISS Comprehensive Conservation and Management Plan (CCMP) (1994) identified habitat enhancement (including wetlands) as an important goal. Tracking reports on the progress of the CCMP implementation note with approval that Connecticut's mosquito ditching maintenance practices are decreasing as its OMWM practices are increasing and that New York State has been phasing out its ditching practices. It was recommended that New York State continue to phase out mosquito ditching and implement OMWM.

The PEP CCMP recommends improved coordination between SCDHS, SCVC, other agencies and departments, and municipalities in maintaining existing mosquito ditches and developing coordinated planning efforts relating to mosquito control in wetlands. The CCMP recommends that OMWM techniques be employed. A no new ditch policy was established, and it was urged that SCVC work cooperatively with all governments and government agencies in planning mosquito ditch maintenance. *Phragmites* control was also emphasized.

Policies have also been established by major federal landholders regarding management of wetlands. For instance, NPS generally (and FINS, specifically) has determined that mosquito ditch maintenance is inappropriate in the park lands. NPS has also identified poor salt marsh quality and the potential for the need for more active salt marsh management as major issues for FINS.

USFWS would also like to eliminate pesticide use in its Refuges, but also maintain its policy of being a "good neighbor" to surrounding communities. Noting the lack of standardized information on OMWM benefits and potential impacts, USFWS has advised that determinations regarding OMWM projects be made at the specific Refuge level, pending the outcome of research targeted at determining the impact of OMWM at other northeast US refuges.

New York State, as shown in the Tables 10-14, has two different regulatory programs and sets of requirements for wetlands protection:

- one for fresh water wetlands as set forth under Article 24 of the New York State Environmental Conservation Law
- one for tidal wetlands under Article 25 of the New York State Environmental Conservation Law.

Both laws and their regulations define wetlands (based largely on vegetation). State laws and regulations also map regulated wetlands, identify permitted activities, and set forth standards for permit issuance.

Two of the more significant differences between the two wetland laws and their regulations are:

- the Freshwater Wetlands Act regulates activities within 100 feet of the edge of wetlands while the Tidal Wetlands Act regulates activities within 300 feet of the edge of wetlands.
- the regulation of fresh water wetlands can be delegated to local municipalities provided the regulations are at least as restrictive as the regulations in effect pursuant to the Freshwater Wetlands Act.

Ditch maintenance activities have been found to be generally acceptable under the Tidal Wetlands Regulations. Other forms of marsh management require further review, and generally are determined to require a permit. Suffolk County has applied for and received general permits for its marsh management activities, including replacement of in-kind water control structures and ditch maintenance. NYSDEC has suggested, however, that it most probably will not issue new general water management permits in the foreseeable future.

The application of pesticides directly to any regulated body of water in New York (that is, "waters of the State") is considered an aquatic application. As such, it requires an NYSDEC Article 15 Aquatic Pesticides Permit. This regulation covers the application of any larvicide to standing water, except for water solely within artificial containers or other, isolated waters not considered "waters of the State." The County maintains such a permit (through SCVC). ULV

adulticides are not applied directly to water, do not target the aquatic stage of mosquitoes, and so do not require an Article 15 permit.

The NYSDEC Article 24 regulations (6NYCRR Part 663) state that the application of a pesticide covered under an Article 15 permit also requires a separate Freshwater Wetlands permit. The County also maintains an Article 24 permit to allow for its fresh water wetland larvicide program. Application of adulticide within 100 feet of an NYSDEC regulated fresh water wetland area requires a permit. NYSDEC has indicated that ULV adulticide applications that take place 150 feet or more from fresh water wetlands will be considered out of Article 24 jurisdiction. The County therefore maintains such a setback on its vector control adulticide applications.

If an application of adulticide over a regulated fresh water wetland is deemed necessary, an emergency authorization can be requested from NYSDEC if NYSDOH has previously declared a Health Threat. The emergency request to NYSDEC needs to present the specific reason the application is needed, with maps delineating the application zone. Emergency authorizations allow actions required to respond to a public health threat that otherwise might have been considered to violate State regulations.

The NYSDEC Article 25 regulations (6NYCRR Part 661) state that the use or application of any pesticide, where otherwise authorized by law, does not require a permit. Thus, if a pesticide is registered in New York State and is applied per the label, a permit is not needed (except if an Article 15 permit is required, for larvicide applications, see above). Application of adulticides over tidal wetlands is generally avoided. If required due to a public health threat, such applications can be made without a specific permit if the product label specifically allows such use of the product over tidal marshes.

Application of pesticides to NYSDEC-owned lands requires NYSDEC permission. This permission can be received in the form of a sign-off on Article 15 permits for larvicide use.

NYSDEC implements several other programs that indirectly affect wetlands:

- Coastal Erosion Hazard Areas Program: actions that may affect mapped coastal erosion zones receive further regulation.

- Use and Protection of Waters (Stream Protection Program): regulates disturbances of stream beds, or excavation and fill of any navigable waterway.

- State Pollution Discharge Elimination Program (SPDES): regulates discharges into surface water by industrial, commercial, and municipal sources, and some residential areas as well, including a ban on the discharge of untreated stormwater to wetlands.

- Water Quality Certification Program: under the Clean Water Act, New York State was delegated the authority to regulate discharges to navigable waters.

- Endangered Species Program: regulates activities that might harm Federal or State listed endangered or threatened species.

- Natural Heritage Program: this program identifies occurrences of rare biota and maps natural communities, and is funded jointly with The Nature Conservancy.

- Wild, Scenic and Recreational Rivers System: designated systems receive extra protections.

NYSDOS developed a State-wide Coastal Management Plan, which established policies that are used by NYSDOS when it reviews Federal and State actions in the coastal zone, subjecting them to a single set of locally-determined criteria. There are now 13 criteria that must be complied with in these "consistency" reviews. NYSDOS can further delegate coastal management authority through the Local Waterfront Revitalization Program (LWRP) (see below).

Another element of the State Coastal Management Plan was the designation and mapping of Significant Coastal Fish and Wildlife Habitats. These sites are further protected, with the intent of preventing impairment. A goal is to restore any such habitats to improve them, where practical.

In 1999, NYSDOS completed a management plan for Long Island Sound, designed to help spur implementation of the State Coastal Management Plan. Protection and restoration of tidal and included fresh water wetlands was identified as a priority. One of the identified important impairments of the mid-Sound coast was ditches installed for mosquito control purposes. Invasive plants, presumably including *Phragmites*, were also identified as a problem.

Recommendation 11 of the Plan was to reach a net gain in quality and quantity of tidal wetlands, and no net loss for fresh water wetlands.

The South Shore Estuary Reserve, a planning effort under the direction of NYSDOS, determined that wetlands were key elements of the biological landscape, and called for increasing the quantity and quality of wetlands, especially tidal wetlands. The means of doing this were identified as primarily being:

- Hydrological modification of formerly connected wetlands
- Restoration of dredge spoil sites
- OMWM
- Establishing protective buffers
- Identifying existing high quality wetlands

NYSDOS and NYSDEC collaborated on the production of *Salt Marsh Restoration and Monitoring Guidelines* in 2000. The document is intended to serve as a framework for New York salt marsh restoration activities, including planning, design, implementation, and monitoring for restoration projects sponsored by municipalities. The goal statements for habitat restoration in New York State are summarized as follows:

- To the greatest extent practicable, achieve functional, community, and/or ecosystem equivalence with reference sites when undertaking restoration.
- Restore critical habitats for priority fish, wildlife, and plant species, including those listed as threatened, endangered, and of special concern by Federal and State governments, and species of historical or current commercial and/or recreational importance in New York State.
- Plan and implement restoration initiatives using a regional perspective to integrate and prioritize individual restoration projects and programs.

- To the extent practical, use historical acreages, proportions, and/or spatial distributions to prioritize habitats from a state or regional perspective.

- To the extent practical, ensure where appropriate that historical acreages, proportions, and/or spatial distributions of priority habitats are restored and preserved.

Two desirable OMWM techniques described in the manual are closed systems and semi-tidal systems. Open systems are not discussed.

The New York State Department of General Services administers all State lands below high tide, and issues any grants, easements, or leases required for any private use of such lands.

Suffolk County does not regulate wetlands currently, although in the late 1970s there was a fresh water wetlands law, which was repealed in 1993.

Nine of Suffolk County's 10 townships have local laws that regulate activities in wetlands; in some cases, the local laws regulate the adjacent area. The Town of East Hampton Trustees regulate wetlands, but it is not codified in the Town Code. Review of the nine Town Codes found the following:

Babylon

Chapter 108 Dredging

A permit is needed to remove any material from any waterway, watercourse or upland abutting or adjoining a waterway or watercourse.

Chapter 128 Freshwater Wetlands

This law is very similar to the New York State Freshwater Wetlands Act. A permit is needed for activities within 100 feet of a fresh water wetlands including:

- draining

- dredging

- excavating

- dumping
- filling
- erecting any roads or structures
- discharging pollutants or effluents

Public health activities are exempt.

Brookhaven

Chapter 81 Wetlands and Waterways

A permit is needed for activities within 150 feet of tidal and fresh water wetlands including:

- draining
- dredging
- excavating
- dumping
- filling
- erecting any roads or structures
- discharging pollutants or effluents

Huntington

Chapter 141 Streams, Watercourses & Wetlands

Article I Filling, Diversion, or Draining of Streams and Watercourse

A permit is needed to:

- fill or divert the course of streams, creeks, or watercourses

- divert any stream, watercourse or creek from its natural course
- drain any pond or impoundment.

Chapter 141 Streams, Watercourses & Wetlands

Article II Freshwater Wetlands

The Town assumes the implementation of the New York State Freshwater Wetlands Act.

Chapter 137 Marine Conservation

Article II Removal or Deposition of Material

A permit is needed to remove/place material on wetlands or watercourses owned by the Town.

Chapter 137 Marine Conservation

Article III Construction or Reconstruction

A permit is needed to construct or reconstruct a dam or impounding structure and docks, piers and pilings.

Islip

Chapter 67 Wetlands and Watercourse

A permit is needed to dig, dredge, excavate, or dump on tidal waters, tidal marshes, fresh water wetlands, coastal wetlands, tidal wetlands, and watercourses.

Riverhead

Chapter 107 Tidal and Freshwater Wetlands

A permit is needed to dig, dredge, excavate, or dump on tidal waters, tidal marshes, fresh water wetlands, coastal wetlands, tidal wetlands, and watercourses and within 150 feet of wetlands.

Shelter Island

Chapter 129 Wetlands

A permit is needed for dredging, disturbing, filling, or excavating in tidal and fresh water wetlands, and within 100 feet of a wetland.

Smithtown

Chapter 170 Freshwater Wetlands

Pursuant to the New York State Freshwater Wetlands Act, the Town assumes the implementation of the Act.

Chapter 138 Dredging

A permit is needed to remove or deposit fill from any wetlands or watercourse.

Southampton

Article VII Regulating Dredging, Docks, Bulkheading and Channels (Board of Trustees)

A permit is required to dredge or deposit material on the bottom of any waters in the Town.

Chapter 325 Wetlands

A permit is needed to undertake open water marsh management measures and to place, deposit, or dredge material in a tidal or fresh water wetland area, or within 200 feet of a wetlands boundary.

Southold

Chapter 97 Wetlands and Shorelines

A permit is needed to remove material from wetlands or to deposit or discharge material on tidal or fresh water wetlands

Suffolk County has a special wetlands regulatory situation, which is the result of local history and practice. This is the establishment of town trustees, who in three cases are entirely separate from other elements of Town government. At this time, towns maintaining separate town trustees are:

- East Hampton: nine trustees, two-year terms

The Trustees own and/or manage waters, lands underwater and adjacent beaches. In discharging their duties as the owners and/or managers of the above, the Trustees have developed policies and regulations designed to improve water quality, increase the productivity of their holdings and protect public rights. These include regulating docks, controlling boat discharges, involvement in shellfish propagation and quality enhancement programs, and designating areas in their harbors for various activities, such as water skiing, mooring, windsurfing, fish trap, and duck blind areas. They review, and must approve, all dredging projects. They review, and must approve, all bulkheads, revetments and other erosion control devices proposed to be constructed, or which may have an impact, on their lands. They have adopted rules governing beach driving and work with the Town Board to coordinate their efforts toward more responsible beach use. The Trustees also own many upland parcels, numerous roads in all areas of Town, and many properties between the ocean and "oceanfront" residences.

- Southampton: five trustees, two-year terms

Duties of the trustees are to preserve public access to the water, uphold the traditions of a maritime community, advise the Town Board on coastal related issues, inform the public of the facts of coastal issues and policy, represent the best interest of the freeholders, maintain and protect surface water quality, regulate dock and bulkhead construction and impacts, promote sustainable harvest of commercial shellfish and finfish, provide a safe marine environment, and inspect all structures built on bay bottom.

- Southold: five trustees, four year terms

Duties of the trustees are the regulation of any activity along the shoreline of the Town and its inland wetlands, per Chapter 97 of Town Code, and to approve moorings.

In the other patent towns, Town Trustees have become subsumed into the Town Boards. Nonetheless, Town Boards will often need to become the Town Trustees to settle certain issues.

Trustee issues have been extensively litigated and are often subject to intense interest on the part of some community activists, even where the practice of trusteeship is largely ignored.

The six approved and adopted LWRPs for Suffolk County were closely reviewed for specifics which could bear on wetlands management.

Town of Smithtown

There are no direct mentions of vector control actions. Indirect policies that may affect wetlands management include:

- Policy 25 states, "protect, restore and enhance natural and man-made resources."
- Policy 25B states, "prevent the irreversible modification of natural geologic forms and the removal of vegetation from dunes, bluffs and wetland areas."
- Policy 35B states, "wetland channels maybe altered only if the action results enhancing the viability of the wetland area."
- Policy 44 intends to preserve and protect tidal and freshwater wetlands and preserve the benefits derived from these areas.

Town of Southold

There are no direct mentions of vector control in the document, with the exception of one reference: when discussing Hashamomuck Pond, ditching was identified as a potential cause of loss of tidal connection, and therefore something that should be avoided. Indirect references to wetlands management and/or vector control activities include:

- discussion of a restoration of 80 acres of diked agricultural land by the US Department of Agriculture, where tidal flow had been lost, on the east bank of West Creek;
- Policy 6.1, which states, "protect and restore ecological quality throughout the Town of Southold;"

- Policy 6.2, which states, "protect and restore Significant Coastal Fish and Wildlife Habitats," noting specifically that actions that destroy habitat values through physical alteration or significantly impair the viability of the habitat (causing a reduction in vital resources or change in environmental conditions beyond the tolerance range of important species) should be avoided;

- Policy 6.3, which states, "protect and restore tidal and freshwater wetlands," where restoration is defined as reconstruction of physical values, adjustment of adverse chemical characteristics, or the manipulation of biological characteristics back to some prior, preferred state.

Village of Greenport

There are no direct mentions of vector control. Indirect policies that may affect wetlands management include:

- According to Policy 12, "activities or development in the coastal area will be undertaken so as to minimize damage to natural resources."

- Policy 44 aims to preserve and protect tidal and freshwater wetlands and preserve the benefits derived from these areas.

Village of Head of the Harbor

The LWRP does not specifically make mention of mosquito management or pesticide use within its boundaries. There are some policies which may or may not be compatible with marsh management.

- On page II-31, the extreme frailty of the Village's beaches, dunes, escarpments, and extensive tidal wetlands is discussed, and a need to protect these assets natural state as best as possible is recognized.

- Page II-52 discusses Village concerns regarding the preservation of its fresh water wetlands and tidal marshes.

- Under Policy 44, tidal and fresh water wetlands, as well as the benefits derived from them, must be preserved and protected.

- The report asserts that fish and wildlife habitats are within the wetlands and marshes of the village. Policy 7 states that "significant coastal fish and wildlife habitats…shall be protected, preserved, and…restored." Policy 7D states that reducing or eliminating these areas for a "regional public purpose" is allowable, with the condition that there is creation of new habitat in a ratio of two to one.

Village of Lloyd Harbor

There are no direct mentions of vector control. Indirect policies that may affect wetlands management include:

- Policy 7 states, "coastal fish and wildlife habitats…shall be protected, preserved, and where practicable…restored so as to maintain their viability as habitats."

- Policy 12 requires that all activities in the coastal area must be undertaken so as to minimize damage to natural resources.

- Policy 24 addresses preventing impairment of scenic resources. This impairment includes irreversible modification of geologic forms.

- Policy 25 intends to "protect, restore or enhance natural and man-made resources which…contribute to the overall scenic quality of the coastal area."

- Policy 44 states its goal is to "preserve and protect tidal and freshwater wetlands and preserve the benefits derived from these areas."

- Chapter 137 of the Town of Huntington Code is discussed. This code section addresses Marine Conservation. It was established to protect and preserve the watercourses, coastal shorelines, tidal marshes and watersheds. This law also regulates the removal or deposition of soils within wetland areas of the Town.

Village of Sag Harbor

There are no direct mentions of vector control. Indirect policies that may affect wetlands management include:

- The Village Conservation Districts (CDs) are described. These were created to preserve the tidal and fresh water marshes found within any one or all of the CDs. The CDs restrict use of the wetlands by permit. The major intent of the CDs is to preserve the water quality of natural areas.

- Policy 6.3 on page III-21 is intended to protect and restore tidal wetlands.

Generally, policies in LWRPs that relate to wetland preservation generally intend to maintain and enhance wetlands with as little activity in them as possible. Although water management for vector control purposes is nowhere mentioned explicitly, it may be that the policies would be interpreted that water management could not occur unless it resulted in "improvements" to the wetlands. Alteration of a wetland can occur, even to the point of total destruction (see Head of the Harbor), although mitigation may be required.

4.4 Source Reduction Summary

The following table summarizes source reduction efforts under the Long-Term Plan, by focusing on the species of concern identified in Section 1.

Table 15. Source Reduction Summary

Species	Source Reduction Efforts	Other Issues
Aedes vexans	Upper salt marsh management	Fresh water habitat manipulation contrary to current State regulations
Anopheles punctipennis	Household efforts	Fresh water habitat manipulation contrary to current State regulations
Anopheles quadrimaculatus	Household efforts	Fresh water habitat manipulation contrary to current State regulations
Coquillettidia perturbans		Fresh water habitat manipulation contrary to current State regulations
Culex pipiens	Household efforts, stormwater structures	
Culex restuans	Household efforts, stormwater structures	
Culex salinarius	Upper salt marsh management	
Culiseta melanura		Fresh water habitat manipulation contrary to current State regulations; habitat often associated with R-T-E species
Ochlerotatus canadensis		Fresh water habitat manipulation contrary to current State regulations
Ochlerotatus cantator	Salt marsh management	
Ochlerotatus japonicus japonicus	Container, tire management	
Ochlerotatus sollicitans	Salt marsh management	
Ochlerotatus taeniorhynchus	Salt marsh management	
Ochlerotatus triseriatus	Container, tire management	
Ochlerotatus trivittatus	Upper salt marsh management	Fresh water habitat manipulation contrary to current State regulations

It is evident from the table that source reduction efforts can reduce populations of many of the species of concern in the County. However, it is also clear that many actions that are allowed in other jurisdictions, such as draining breeding areas and otherwise manipulating fresh water environments, are not permitted under New York State regulations, in order to preserve these environments. In nearly every case, environmental benefits associated with this general rule are clear. SCVC has interest in reported re-evaluations of New York State wetlands regulations that are said to be occurring within NYSDEC, and would be willing to participate in such efforts, as may be allowable.

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5. Biocontrols

5.1 Introduction

Biocontrols are another alternative for the control of mosquitoes. Biocontrol involves the use of organisms, invertebrate and vertebrate, predator and parasite, some of which have been discussed in sections above. One possible advantage of biocontrol agents is potential host specificity, which implies minimal impacts to non-target species and to the environment. A good example of host specificity is where an introduced organism parasitizes only the target organism, as certain wasp species do with particular crop pests. Such biocontrols would have limited to no impact on non-target species. However, specificity also tends to limit the market for any one biocontrol. In many situations, especially in agriculture, there are a number of potential pest species, each one of which would require a specific biocontrol. This specificity and the occasionally large start-up costs deter commercialization and application of biocontrol agents. In addition, other problems include the generally narrow pest control market and, for the user, increased outlays of capital and the associated training required for personnel.

Advantages of biocontrols are generically said to be:

- Reductions in chemical inputs to the environment
- Little or no effect on beneficial and non-target organisms
- Organisms may naturally be a part of the ecosystem, and only require augmentation to reduce pest populations to the desired level
- Possible recycling or establishment of biological control methods to permanently reduce mosquito populations

Disadvantages of biocontrols have been described as:

- Host specific – effective against only one or a few species
- Mass production is difficult
- Generally more expensive initially than conventional methods

- Require trained personnel to assess conditions under which they can be used effectively
- Generally, more difficult to use effectively than conventional pesticides

Most mosquito biocontrols are not species specific. They tend to target all mosquitoes, although they may be more effective on some species as compared to others. In addition, due to the boom-bust nature of most mosquito hatchings, many biocontrols cannot subsist entirely on mosquitoes. This means they have non-target impacts, which more targeted biocontrol-pest situations avoid.

Biocontrols are introduced into the mosquito habitat through two basic procedures: inoculation and inundation. Inoculations introduce organisms in relatively small numbers, and the introduced organisms reproduce and maintain themselves in the habitat. Population levels may eventually reach equilibrium with the pest population and, thus, provide some long-term control.

Inundation involves the release of large numbers of the biocontrol organism, which is usually a parasite or invertebrate predator, with the aim of immediate reduction of the pest population. Because it is not anticipated that the biocontrol will establish itself in the environment, several inundative releases may be necessary to control the target mosquitoes. The sequence must be repeated if a new brood appears.

Biological control methods fall into six categories:

1) Vertebrate predators (fish, birds)
2) Invertebrate predators (insects, flatworms)
3) Pathogens (bacteria, protozoa, fungi, viruses)
4) Parasites (nematodes)
5) Autocidal (genetic) (not discussed here)
6) Botanicals (plants)

Biocontrols using introduced species have a long history of not achieving desired goals, and in resulting in unintentional impacts that are sometimes worse than the problem that was intended

to be addressed. This strongly suggests that extreme care be taken, especially in sensitive settings such as the County's fresh water wetlands, before steps are taken to alter the ecosystem through biocontrols to reduce mosquito numbers.

Nonetheless, some key potential biocontrols are discussed below.

5.2 Vertebrate Predators

Biological control includes the use of many predators, such as dragonfly nymphs and other indigenous aquatic invertebrate predators, including *Toxorhychites spp.*, a predaceous mosquito that eats mosquito larvae and pupae. However, the most commonly used biological control adjuncts are mosquito fish, *Gambusia*. Differences of opinion exist on the utility and actual control benefits derived from *Gambusia* implementation in a mosquito control IPM program. Reports range from excellent control to no control at all. Recently, concerns have been raised over placing *Gambusia* in habitats where other native fish species may become threatened. Care must be taken in placing this species in areas where endemic fish or other species may be impacted. For that reason, the County has considered using fathead minnows as an alternative. Fathead minnows are not native to Suffolk County, but they are ubiquitous and well-established in many County fresh waters. Still, their use needs to be carefully considered to ensure that no negative impacts result.

It can be very important to preserve environments that have previously been predator free. Vernal ponds are environments that serve important ecological roles because they generally do not support many predators. Natural examples of these pools are usually fed by ground water or snow melt. On Long Island, for example, the ground water table tends to be higher in spring than in other times of the year. This results in vernal pools often drying out at certain times of the year (often summer or fall) and so aquatic life using these ponds needs to find some means of adapting to this situation. One common strategy is to escape the pond environment altogether. Thus, these ponds are often rich in larval forms, and do not contain many mature aquatic life forms. Few fish, for example, can survive the loss of aquatic habitat, and so these ponds host various invertebrates (often including mosquito larvae), and, especially, amphibian larvae that have developed specialized life cycles so as to exploit this niche, and would probably be preyed upon by any fish. This makes it essential that fish not be introduced where key species

reproduce. Storm water management structures such as recharge basins often serve as admirable vernal pool substitutes in suburban settings. In seeking to control pestiferous mosquitoes, SCVC needs to ensure that it does not introduce fish into previously predator free environments that support amphibians and invertebrates that may be less noxious than mosquitoes.

Birds and bats are often promoted as potential biocontrol agents for mosquito control. While both have been reported to eat adult mosquitoes, they do not do so in sufficient amounts to impact dispersed mosquito populations (effective predation can occur as populations emerge or during mating periods, when the mosquitoes tend to swarm). Mosquitoes provide such a small amount of nutrition that birds or bats expend more energy pursuing and eating dispersed mosquitoes than they derive from them, and so cannot be a primary food source. Additionally, with mosquito flight behavior being crepuscular (most active at dawn and dusk), they are not active during the feeding periods of most birds. While bats are active during the same time periods as mosquitoes, they cannot reduce the massive numbers of adult mosquitoes available.

Purple martins consume large numbers of flying insects, and it was reported that each bird can consume 2,000 mosquitoes a day. Research on the stomach contents of purple martins found that they actually appear to eat much bigger insects (beetles, moths, butterflies, dragonflies, and larger flies). There were no signs of mosquito consumption, and apparently no factual basis for the original report.

There are approximately 45 species of bats in the US. Most bats feed on insects, and so bats are touted as insect controls, especially for pests such as mosquitoes. An oft-quoted figure is that bats eat 600 mosquitoes in an hour. Several large scale efforts have been made to promote bat colonies to reduce mosquitoes. However, the basis of the claim was a limited experiment where starved bats were set in a mosquito-filled room, and ate an average of 10 mosquitoes each over one minute. Extrapolation from this experiment for night-time catch rates of 5,000 mosquitoes per bat are not credible. Although bats are opportunistic feeders, mosquitoes apparently make up only a very small percentage of their diet under most conditions. Stomach content analyses have found that more typical fare is moths, flies, caddisflies, and leafhoppers (the little brown bat), or beetles, such as ground beetles, June bugs, cucumber beetles (big brown bat), or moths and beetles (Mexican free-tailed bat). Other species apparently will opportunistically prey on mosquitoes, but usually only when they are in “swarms.” In must be understood that increasing

bat populations carries added risk of bat-associated rabies. In the last 40 years, most human cases of rabies in the US have resulted from bats. Rabies is almost always fatal, and the CDC recommends "prevention of human exposure" to bats as a means of reducing the risk of catching the disease. Sampling is Suffolk County has found that five percent of bats tested for rabies are positive for the disease.

It seems that predators touted as mosquito catchers may prefer to consume larger prey that offer greater reward for the capture effort. In addition, since consumption of mosquitoes will not be capable of sustaining any augmented predator population (especially if control of brooding mosquitoes is sought), the ecosystem must have additional resources to support the predators. This may mean dislodging some other local predators from their ecological niche.

5.3 Non-vertebrate Control Agents

The yeast-like fungus *Lagenidium giganteum,* has been used for mosquito control in still water environments. It attaches to and penetrates the mosquito larvae, then grows inward, eventually filling the body cavity, causing death. It is then released, where it can form more zoospores that can infect other larvae. The parasitic nematode *Romanomermis culicivorax*, the pathogenic protozoan *Nosema algerae*, and some non-digestible algae have been examined as biocontrol agents by university, government (USDA), and by mosquito control organizations, with mixed results.

Another group of biocontrol agents with promise for mosquito control is predaceous copepods. Copepods are easy to rear and to deliver to the target sites in the field, and they generally perform well when used with pesticides. However, they have not been shown to provide the degree of control that comes with other biocontrols such as fish. Copepods must multiply to effectively attack mosquito larvae populations, leading to a lag time between inoculation and effective control. There is some County interest in developing a copepod program in Suffolk County as some species may be effective for long-term control in catch basins. New Jersey is actively researching this biocontrol method, however. In areas with seasonal rain patterns, brine shrimp have also shown promise as similar larval predators.

Rotenone and pyrethrum are plant products that have a long history in pest control. Pyrethrum is discussed in Section 7, Adult Control. Substances released from bladderwort (*Utricularia*),

stonewart (*Chara*), and duckweed (*Lemna*), are known to be toxic to mosquito larvae. Extracts from an alga (*Elodea nuttallii*) and a sage brush (*Artemisia cana*) are highly toxic to immature mosquitoes. Data have not been collected as yet regarding their efficacy on different mosquito species and selected non-targets. The mode of action for most of these toxicants remains unknown.

Dragonflies are predatory insects that catch midges, mosquitoes, small moths, and even bees and butterflies. The use of dragonflies to control mosquitoes has been used in the town of Wells, Maine, where dragonfly augmentation was incorporated into their mosquito control program. The Town raises and sells dragonfly nymphs to local individuals who want to use them on their property. Dragonflies are difficult to rear in the laboratory for release, and they have a very long life-cycle, meaning populations cannot be quickly increased. In addition, dragonflies are free to fly about and therefore cannot be contained in the area where control is desired. Dragonflies do, in fact, eat mosquitoes, and, therefore, reduce local mosquito populations to some extent. The determination of the effectiveness of dragonflies in Wells is complicated by the Town's concurrent use of Bti. The apparent success of the dragonfly release program could be due to larviciding. In New York State, there is only one species of dragonfly that utilizes salt marshes. Some dragonflies in fresh water marshes synchronize hatching to coincide with springtime mosquito emergences. This is one of the few instances of any known direct ecological links between mosquitoes and another species.

6. Larval Mosquito Control

6.1 Background Information

Larviciding is a general term for killing mosquitoes by applying natural agents or commercial pesticides to control the larval and pupal stages of the organisms. Since both of these life stages are exclusively found in aquatic environments, larvicides are always applied to water. Larvicide treatments can be applied from either the ground or air. Larviciding, originally implemented as a malaria control procedure in the early 1900s, has become a mainstay of mosquito control over the years. As populations of larval mosquitoes are more concentrated in limited geographical areas, the portion of the environment that needs to be treated is less than when targeting adult mosquitoes.

Safely altering aquatic environments, even temporarily, for the purpose of controlling mosquitoes requires a good working knowledge of both the target species and larvicides. Mosquito control now approaches prescription applications, where a competent operator will apply one or more larvicides in an environmentally sound manner under a given set of conditions to address particular species and ages of larvae.

The Long-Term Plan proposes to use three biorational products as its primary larvicidal treatments. These three products, *Bacillus thuringiensis var israelensis* (Bti), *Bacillus sphaericus* (Bs), and methoprene, have been shown through the quantitative risk assessment to have no quantitative risk of impacts to human health, and apparently no significant or substantial risk of impacts to the environment.

These conclusions are supported by independent scientific experiments conducted by the Long-Term Plan, and a rigorous review of the scientific literature. It is a general objective of the Long-Term Plan to avoid the use of pesticides, whenever possible. It is a basic tenet of IPM that an excessive dependence on pesticides is not wise from a programmatic point of view. An excessive reliance on pesticides can make a program vulnerable to control failure. For instance, logistical problems or weather conditions may prevent the application of pesticide in all areas where they are needed and at the proper times. Development of resistance to pesticides to the targeted organisms can be a problem. In addition, if a widely used material is found to have

unacceptable impacts, or if it becomes unavailable due to market forces, a program that is overly dependent on that material can find itself without viable options. Sound management principles dictate that pesticides must be just one part of a comprehensive control program. These management principles result in a long-term plan that emphasizes water management as a means of reducing larvicide applications. Scientific surveillance measures are the means of ensuring that larvicide applications are truly necessary. Surveillance data analysis to establish site-specific values for dipping results may allow for further reductions in larvicide applications. These measures should be taken despite the Long-Term Plan's determination that larvicide applications have no apparent increase in risk of human health or environmental impacts of substance or significance.

Fresh water wetlands require special consideration for any pesticide treatment. These environments are more diverse than salt water mosquito breeding sites, and have the potential to be more sensitive to perturbations. Most of the species of special concern in the County are found in or near fresh water wetlands. Therefore, the County will, over time, through consultation with State, County, and town natural resource staff and other interested parties, develop GIS determinations of the fresh water areas that require more nuanced approaches to treatment decisions. A focus will be on the identification of vulnerable species, and to determine the points in their life histories that may make them more susceptible to potential impacts from vector control operations, and then to determine what modifications of vector control activities can be made to mitigate the potential impacts. For instance, because of special reproduction requirements for certain species, spring or early summer pesticide treatments may be counseled against. Methoprene, for instance, has been shown to impact frog eggs and very early stages of tadpoles. Where these stages of amphibians exist, methoprene use should be curtailed unless absolutely necessary. In other instances, early morning or evening applications may be preferred in order to avoid knock down by sprays of day-active insects. These plans may become customized for particular settings. An expansion of GIS capabilities in the County may facilitate this approach. As inventories of the wetlands and the special habitat and other needs of important species are ascertained, special research conducted on behalf of the County may be able to craft modifications of its standard operating procedures to reduce the chances that any negative environmental impact will follow from treatments. As an important example of this, following consultation with NYSDEC, SCVC has removed all tiger salamander habitats from its

larvicide list, to ensure that no possible impact from these pesticides to this rare species can occur.

6.2 Surveillance

All treatment decisions will be made on the basis of scientific surveillance to determine the need for the treatment. In the case of larvicide applications, appropriate surveillance requires sampling for the presence of larvae. Although standardized sampling methods have been developed (and discussed in the scientific and technical literature) for larval sampling of all kinds, the results of the testing are almost all sampler-dependent. Dipping, the standard open water technique requires learning to approach the water systems so as not to startle the larvae, and/or to comb through fringing vegetation. This increases the likelihood that different samplers will capture different numbers (and, potentially, different species) of larvae. Catch basin sampling has not been as standardized, but also seems likely to be dependent on some degree of sampler skill in identifying the proper level to scoop larvae from, and to execute the sampling run consistently. Some programs report larval sampling quantitatively, and report the results as measures of effectiveness or as a relative risk measure; it is far from clear that the necessary accuracy and precision to conduct that kind of analysis is associated with the sampling techniques. For example, on a salt marsh, breeding often occurs in small potholes at or near to *Spartina patens* (salt hay). Numbers of larvae per dip under these conditions can be extraordinarily high. However, the same number of larvae, if dispersed through a panne area, will generally have a lower number of larvae recorded per dip because of the larger area over which the larvae might be spread. The potential for impact from adult mosquitoes (and therefore, the evaluation of the need to act) would be the same in both examples, although the number of larvae recorded per dip would be much less for the panne setting as compared to the pothole setting. Thus, generally, for salt marshes presence/absence and the proportion of the marsh that appears to be breeding (due to the extent of the tidal flooding) are much sounder means of determining if a need for larval control exists.

SCVC has had good experience using a larval dipping index at Wertheim National Wildlife Refuge. However, at that site, the same sampling crews tended to repeat measurements at consistent locations. The particular trigger value set for Wertheim larviciding was determined post-facto; analysis of sampling data showed that if a particular value for the trigger were to be

used, it would eliminate a certain number of larval treatments. Prior to setting the trigger value, SCVC had determined that the neighboring community might not suffer from excessive numbers of mosquitoes if some of the larviciding treatments had been reduced at Wertheim. As treatments could not be entirely eliminated, using a particular mean number of larvae per dip that resulted in a reduction of larvicide treatments by approximately 25 percent should not result in excessive community impacts. This is somewhat of a unique situation, since the distance from the treatment sites in the National Wildlife Refuge is greater than the distance is for most salt marshes to surrounding houses. Much of the larval sampling in the SCVC program is in response to complaints, and so necessarily requires sampling in disparate areas. This tends to reduce the opportunities to establish similar field-based trigger values.

Therefore, generally SCVC will continue to rely on absence/presence tests of larval habitats at this time. Absence/presence determinations are the most common triggers for treatment in the region, with, for example, most New Jersey programs following similar guidelines. Qualitative assessments by samplers of relative population densities (none-some-many-throngs) will be used as a determinant of apparent populations. Samplers will also record actual numbers of larvae, as possible, per dip. For the identified breeding locations, data analysis of these numbers will be pursued, and it may be that site-specific triggers that appear to lead to reasonable reductions in larviciding frequencies can be developed over time. Samples will be collected for laboratory speciation, as well.

Until site-specific triggers are established, however, the identification of a potential mosquito problem will determine the need to control larvae. Potential mosquito problems will be defined in terms of complaint history, close association with residential or recreational settings, or disease history or other risk factors, and the presence of human-biting mosquito larvae. The presence of human-biting mosquito larvae is a determination made most often by observations through sampling with identification of the larvae as a pest species by field crews, or by the subsequent laboratory analysis of the returned specimens.

Permanent and transient fresh water breeding habitats have also been identified and catalogued by SCVC (see Figure 1, above). The permanent water sites are visited on a regular basis. Transient water sites, which are not as extensive in Suffolk County due to the high permeability of the soils (generally), are sampled following significant rainfalls. History dictates the kinds of

rains likely to produce breeding. It has been suggested that establishing a trigger value for permanent water sites is possible; again, this needs to be determined through correlation of the number (and kinds) of larvae that later result in adult mosquito population problems. These computations are likely to be site specific, as larval density is not consistent across habitats, and so the selection of a County-wide trigger value would be arbitrary.

6.3 Mosquito Problem Identification

There are four types of areas where SCVC may apply larvicides. They are:

- catch basins and other, mostly underground, storm water control structures;
- sites identified by complaints (mostly household-institutional sites);
- breeding areas within marshes that are aerially larvicided;
- breeding areas that are not within marshes that are aerially larvicided.

Storm water structures

Some storm water structures have been identified as potential breeding problems by SCVC through surveillance work. There are approximately 10,000 such structures that currently monitored to determine if a need for treatment exists. Records indicate that 7,601 applications have been made over the past six years (2000 to 2005), predominantly using long-lasting methoprene briquets. SCVC proposes to expand its surveillance of catch basins so that another approximately 40,000 sites are evaluated in the first year of the Long-Term Plan. It is estimated that there are 100,000 catch basins in the County, although the actual amount is unknown. These sites will be prioritized first by age. An assessment of some systems by Cashin Associates suggested that older systems, due to a greater likelihood of poor maintenance, are more likely to hold water and therefore breed mosquitoes. Where possible, maintenance records and plans of appropriate agencies, typically, SCDPW or town and village highway departments, will be accessed prior to the surveillance effort. Recent cleaning generally means that the system will not hold water, and cleaning plans will mean that treatments will be wasted as the larvicide material would be removed in cleaning the basin. If the basin shows signs of breeding, it and all connected basins will be treated to limit the risk of potential mosquito disease transmission. *Cx.*

pipiens is the dominant mosquito species in catch basins, and has been identified as the major WNV disease vector for Suffolk County.

Open water systems, such as recharge basins, without histories of treatment will be assessed similarly to environmental sites identified in complaints.

Sites identified by complaint

Most complaint call investigations are easily resolved by identifying household breeding sites, and remediating them. These kinds of problems, such as gutters that retain water, open containers, bird baths, wading pools, pool covers, etc., can be treated by removing the water (and the cause of the water accumulation, such as turning a bucket upside down, or recommending gutter clean out) without needing any pesticide applications. In some situations, the household mosquito source is too large, such as a poorly maintained above or below ground pool or impossible to treat like a cesspool. In those instances, treatment with a larvicide may resolve the immediate problem, and allow time to investigate for long-term management of the underlying problem.

In other complaint situations, the source of the troubling mosquitoes may appear to be an environmental setting, e.g., a local wetland area. If the wetland is a known breeding area subject to regular surveillance, then the appropriate problem determination procedure will be followed, as detailed below. Such sites will be identifiable by the vector control crews as they respond to complaints in areas that they generally are familiar with from surveillance activities. In addition, all known breeding sites are mapped and available through GIS, allowing the team to quickly identify in the field whether the complaint is originating from an existing breeding site. If the site is not a known breeding site, then sampled larvae will be brought to the laboratory for official identification, and follow-up at the site shall be undertaken by senior level staff.

Options available on this follow-up include minor water management to resolve a drainage or fish access issue, larvicidal treatment, or assignment to a follow-up surveillance list. The determination as to whether to treat the site will be through evaluation of ecological issues and the degree of seriousness of the problem. If the site is obviously an area where species of concern are likely to be found, or the life cycle of a particular organism of interest suggests that at particular times of the year it may be more at risk, or it is in an area (such as the Central Pine

Barrens) predetermined to be of environmental concern, treatment may be deferred, unless the scope of the problem appears to require immediate action. The senior staff will annotate the SCVC GIS with appropriate treatment trigger information, including quantitative or qualitative larvae presence factors, time of year, or other issues of note. As described above, these kinds of determinations will be generated through consultation with State and town natural resource staff, and interested parties, and through targeted research sponsored by the County.

Aerial larviciding sites

Sites that are considered for aerial applications of larvicides are those that are too large or inaccessible for ground application and breed mosquitoes consistently and persistently. Almost all aerial larviciding occurs at salt marshes. There are approximately 4,000 acres of salt marsh that receive aerial larviciding at this time. A major focus of the water management plan is to substantially reduce this acreage. However, it will require time to implement the physical changes to the marshes that will result in better water management and predation by native fish on the larvae, if such modifications are deemed to be appropriate. It has been the experience of other jurisdictions that progressive water management generally leads to elimination of the need for regular larviciding.

Until those projects have been undertaken, the sites will be monitored weekly by SCVC crews. Testing in the salt marsh will be on a presence/absence basis, with identification of the larval stage included to guide pesticide choices. Almost all mosquitoes found on these marshes at the peak of the season are *Oc. sollicitans*, although other species dominate in spring (*Oc. cantator*) or are a substantial presence later in the season (*Oc. taeniorhynchus*), and others are sometimes detected (most often through laboratory identifications). Use of GPS equipment will allow for good determinations of the portion of the marsh that is breeding. Field observations regarding the intensity of breeding will also be useful for decision-makers. In addition, the state of the tide and the status of water on the marsh may be used in making treatment decisions. If weather forecasts suggest the breeding habitats will dry down prior to any adult emergence, then the need to larvicide has been removed.

It may be that a careful analysis of treatment histories and subsequent adult mosquito infestations suggest that a certain amount of larvicide treatments can be eliminated for some of the marshes.

Then analysis of larval survey records may help determine some kind of threshold value for each particular marsh, probably based on a mean number of larvae per dip. However, until such values are established, SCVC will continue to rely on presence/absence determinations as a sign of excessive breeding in the salt marshes.

Breeding sites not aerially larvicided

There are other breeding locations that are regularly monitored by SCVC. These are wetlands that do not require aerial treatments, either due to their small size or relatively minor mosquito problem. The kinds of mosquitoes that can be expected to be found at these sites have been well determined over time. Therefore, field crews can often make treatment decisions based on sampling results, and efficiently treat any problem that is brewing.

Fresh water sites on this list are good candidates for reassessment of routine treatment measures. It will be important to factor into the decision-making regarding such sites that the control of bridge vectors probably plays an important role in the prevention of EEE County-wide, and so it is unlikely that major breeding sites for known EEE vectors will be allowed to flourish without intervention. Nonetheless, as with the frequency of larviciding in certain salt marshes, some of these fresh water sites may be places where treatment patterns can be altered to ensure that there are no non-target impacts to important elements of the ecosystem.

6.4 Larval Treatment Selection

The choice of methods for larval control is based on several factors:

- Species of mosquito present
- Kind of habitat to be treated
- Stage of larvae present
- Efficacy of the considered treatment
- Residual effects (potency and duration)
- Potential environmental impacts of the considered treatment

- Resistance management

The selected larvicides, Bti, Bs, and methoprene, all tend to score well in each of these areas. Some other pesticides do not rate as well, and so their use was not preferred.

Most larvicides are effective against all mosquitoes. This is true although the range of action of larvicides has been generally reduced compared to that of broad spectrum insecticides. Methoprene, as an example of a more modern pesticide with a narrow range of action, generally is lethal only against members of Diptera (true flies), especially at the low concentrations (parts per billion) associated with applications. Diptera includes mosquitoes.

Species composition is important for gaining some understanding of breeding patterns. For example, if the larvae belong to a univoltine, brooding mosquito, generally long acting pesticides would be wasteful as there will be no further breeding once this episode passes. For multivoltine, steady-breeding mosquitoes, it is not important to know what stage is currently dominant, as breaking the breeding cycle is more important. For brooding, multivoltine mosquitoes such as *Oc. sollictitans*, knowing what stage the current brood is in becomes very important, so as to disrupt what may be a large emergence of a brood. Therefore, identifying the species of mosquito that is breeding is important.

Certain larvicidal compounds are effective only in open water; some lose effectiveness if there are too many particulates in the water; some are best for permanent bodies of water, while others have extensive environmental requirements in order to be effective. One extreme example of requiring certain conditions for larvicidal action is brine shrimp, which are used in certain kinds of agricultural fields in California, and need to undergo special cycles of drying, wetting, and to experience certain temperatures to hatch and then consume mosquito larvae. Another example is surface films, which are best suited for prevention of breeding over expanses of open water that are relatively still and do not have extensive amounts of vegetation. Where such conditions do exist, a relatively small access point can allow for coverage of large amounts of acreage, because the products tend to spread very well. Environments that are well suited for such products are relatively few in Suffolk County, and so the use of monomolecular surface films has not been recommended as part of the Long-Term Plan.

Bti and Bs need to be ingested to be effective. This limits their utility to Stage I, Stage II, and Stage III larvae. In the salt marsh, Bti seems most effective on stages I and II, when the marsh is very wet, and when temperatures are relatively low. If these pesticides are considered for use, then they either need to be applied to situations when they will eventually choke off further breeding, or where they will directly affect most of the mosquito larvae in the area.

Applying products that will not achieve the desired end is not only wasteful, but violates the prime tenet of IPM to avoid pesticide use wherever possible. Bs, Bti, and methoprene have all been demonstrated to be generally effective, although there are some limitations due to acceptable environmental conditions for Bti. Testing of the compounds has shown order of magnitude or more reductions of potential populations due to their applications. Suffolk County-specific data showed that adult salt marsh mosquito populations were reduced by nearly another order of magnitude when methoprene was added to a Bti-based larviciding program in 1995. Analysis of the County's larviciding records shows that methoprene was used in approximately half of the applications, and Bti and Bs in approximately half, as well. Some of the other compounds SCVC has been urged to adopt do not have clear demonstrations by disinterested parties that they are effective.

A certain degree of residual effect for a targeted pesticide appears to be a favorable characteristic. However, the lessons of the very long-lived post World War II pesticides is that compounds that degrade slowly may have unexpected environmental impacts. Therefore, most of the longer-lived larvicides are that way because of natural actions (Bs, as a bacteria, propagates in nature under certain conditions) or because the application has been treated to slow release of the pesticide to the environment from its packaging (time release formulations). This eliminates the need for the program operator to be as precise in the timing of applications as might otherwise be necessary. It also allows for the pesticides to impact species effectively when not all eggs mature at the same time (as with *Cx. pipiens*). A danger of time release formulations is that the released concentration may not be high enough to effectively kill all of the dosed larvae. This can lead to resistance in such partially-treated populations. Testing of methoprene time-release formulations shows this is not a problem with these products. Potential environmental impacts associated with control measures are important factors in selecting agents to be used. The selected agents, Bs, Bti, and methoprene, have long research histories that

generally find no to little non-target impacts. The ecological risk assessment analysis conducted for the Long-Term Plan found little likelihood of ecosystem impacts from these chemicals, and research conducted as part of the project (such as invertebrate surveys of larvicided marshes and follow-up work associated with the Caged Fish experiment) also did not detect larviciding impacts to marine invertebrate communities.

Other larval controls do not have as robust histories of research for potential impacts. Particularly, the monomolecular films generally have fewer independent studies to determine any impacts to non-target organisms. Monomolecular films are not as targeted control mechanisms as the selected agents, and so may have a potential to result in non-target effects.

Some controls have been extensively studied, but have been found to potentially have greater impacts on the environment than the selected agents. Temephos falls into this category. However, because temephos does not have a New York registration, it did not receive extensive consideration for inclusion as a larvicide for the Long-Term Plan.

One other alternative to pesticidal larval control has been touted. New Mountain Innovation Company has produced a Larvasonic acoustic device, which is expected to kill larvae using sound energy. The device gives off sonic energy as a short (less than 15 seconds), minimal energy burst of about 400 watts that causes air spaces within each larvae to resonate violently enough to kill them by disrupting internal membranes and organs. There are several adaptations of the technology:

- a hand-held unit, about the size and shape of a weed-eater, for ditches and wetlands,
- a canal-pod unit to be towed behind a boat in canals, and
- a storm drain unit.

These devices are expensive ($4,000 and up) and are limited in their "killing power" to a range of three to 25 feet in diameter, corresponding with the model being used. In addition, controlled laboratory testing against other closely related non-target aquatic insects or other invertebrates has been limited; and there has been only limited field testing so far. Testing against larvae in a wading pool found the machine killed *Cx. pipiens* larvae better than expected, but did not

succeed with *Oc. triseriatus* larvae. Perhaps the most important consideration limiting the use of the Larvasonic device is the fact that it only kills the larvae present at the time the device is used. In this way, it is similar to the short-acting biological larvicide, Bti. Given that there are few ecological reasons to limit the use of Bti, it is not clear that there is any environmental benefit to be gained from the use of this costly device. It may have uses in permanent installations such as in sewage treatment plants. The main advantage of the Larvasonic is that it is not regulated as a pesticide. This may make it an attractive alternative for small-scale programs that are not prepared for the efforts needed to comply with pesticide regulations.

Resistance to control is always possible. One reason for the County to use multiple larvicide products is to allow for resistance management. The County tends to alternate between Bti and methoprene in salt marshes, for example. Bti is effective with Stage I, Stage II, and Stage III larvae, so when development is slower in spring and later summer, Bti is preferred. Methoprene prevents larvae from developing, and is a contact pesticide; so it is effective for all stages of larvae, especially late stages. It is used when larvae are developing quickly, as the lag between detection of larvae in the marsh and treatment with Bti in summer could result in ineffective treatments, as no susceptible organisms would remain because they had all become Stage IV or later organisms. This suggests that methoprene may be a more effective larvicide overall than Bti, and indeed County records show large improvements in larval control efficiencies when methoprene was introduced in 1995. However, reliance solely on methoprene could run a considerable risk of developing resistant mosquitoes, by eliminating all mosquitoes except those that methoprene does not kill. Bti uses five distinct toxins to kill mosquitoes; it is generally believed that so many toxic compounds slow resistance development, and so from that standpoint Bti has advantages. It has been SCVC's experience that using both these materials has resulted in a more effective program than would be possible if only one of either is used. By having Bti and methoprene available for use, SCVC is able to use each of them under the conditions where they are more likely to be effective.

The County will also use a duplex formulation of Bti and methoprene in summer when generations appear to be overlapping, or development is especially rapid. This can also aid in resistance management to either material should any occur, since it is unlikely that mosquitoes can develop resistance to both products simultaneously.

In sum, the selection process found that the pesticides Bs, Bti, and methoprene should be chosen for use due to the advantages in control that they present, and because they do not have certain disadvantages associated with other larval products.

6.5 Selected Compounds

Bacillus thuringiensis israelensis (Bti)

Bti is a naturally occurring soil bacterium used as a microbial pesticide. Microbial pesticides are comprised of microscopic living organisms (e.g., bacteria, fungi, protozoa) or the toxins produced by these organisms. Bti is used to control the filter feeding stages of mosquito, black fly, midge, and fungus gnat larvae. Granular and liquid formulated products can be applied through ground or aerial application. Bti is commonly registered under the trade name VectoBac and Teknar. These materials consist of bacterial spore, rather than live bacteria, and must be ingested by the larvae to be effective.

Bti's selectivity in terms of its ability to target the larvae of certain insect species, particularly mosquito and black fly larvae, is attributable to a variety of factors. Bti produces five distinct types of exotoxins. Targeted insects are less likely to build up resistance to Bti because each of the five produced toxins varies to some degree in its mode of toxicity. Alkaline conditions in the larvae's gut, generally corresponding to a pH of seven or greater, are required to activate these exotoxins. Specific enzymes must also be present in the gut to cause activation. In addition, distinct chemical receptors must be present in the plasma membrane of the gut to encourage binding of the exotoxins. Mosquitoes that are most susceptible to Bti include species in the genera *Aedes* and *Psorophora*. *Anopheles* and *Culex* are also susceptible to Bti, but generally higher application rates are required.

The World Health Organization (WHO) has concluded that because of the specific nature of the mode of action of Bt products, they are unlikely to pose a health risk to humans or other non-target animals, as long as they are free of exotoxins or other non-Bt microorganisms. This conclusion is supported by the lack of reports of adverse health effects in workers who manufacture Bt products. The protein involved in the pesticidal activity of Bt (Cry3Bb1) has been tested and shown not to produce toxicity in mammalian species.

USEPA does not consider Bti to be a risk to non-target organisms, on principle. Nonetheless, the behavior of Bti, and Bt strains in general, has been fairly well studied. The length of time that Bti remains effective against insect larvae varies, depending primarily on the species and behavior of the larvae, environmental conditions, and water quality. In general, Bti is effective from one to seven days after application. Because Bti is used predominantly in aquatic settings, its response to light has not been extensively studied. However, UV light in the range of 300 to 400 nm, falling within the wavelength range of sunlight, has been shown to inactivate both spores and exotoxins of Bt. Bti toxin can last for a few months in the soil and has an above-ground half-life of one to four days on plant surfaces. As a result, exposure to most above-ground non-target organisms is expected to be minimal. In aquatic environments, Bti has a tendency to bind to particulate matter in the water column and settle out on the bottom. When adsorbed to particulates in the water column, Bti is too large to be ingested by insect larvae. Once settled on the bottom, Bti is not available for consumption by targeted mosquito and black fly larvae which reside in the open water column or at the water's surface. Thus, the efficacy of Bti may be limited in aquatic systems with a large amount of particulate matter.

Bti, as is the case with Bt strains in general, does not colonize or cycle (reproduce and persist to infect subsequent generations of pests) in the magnitude necessary to provide continuing control of target pests. The bacteria may multiply in the infected host, but bacterial multiplication in the insect does not result in the production of abundant spores or exotoxins. Once larvae die, few or no infective units are released into the environment.

There is some evidence of Bti effects to non-target aquatic dipterans that include midges (Chironomidae), biting midges (Ceratopogodinae), and dixid midges (Dixidae), which are commonly associated with mosquitoes within the aquatic environment. These organisms are taxonomically similar to mosquitoes and black flies and can possess the gut pHs and enzymes necessary to activate Bti's delta-exotoxins. Adverse effects to these groups, however, have only been noted at dosages 10 to 1,000 times greater than the application rate specified for mosquito control.

Overall, USEPA has concluded that Bti does not pose significant adverse risks to non-target organisms or the environment, especially since rates higher than those used for vector control are

needed to produce any adverse effects. Bti has been used by SCVC since 1982. SCVC was one of the first programs in the US to use this material on a wide scale.

Bacillus sphaericus (Bs)

Bs, as with Bti, is a naturally occurring bacterium used as a microbial pesticide. Bs is found naturally in soil and aquatic environments. Commercial formulations utilizing Bs (e.g., VectoLex) consist of living bacterium that produce spores. Granules that contain the Bs are mixed with water and other substances, and then sprayed from the air or from the ground.

Bs spores produce two delta-exotoxins that are toxic specifically to mosquito larvae upon ingestion. Similar to the mode of action of Bti, Bs exerts toxicity through the release of the exotoxins upon ingestion by mosquito larvae, which results in the disruption of gut activity and ultimately leads to death. The selectivity of Bs is attributable to the fact that certain gut conditions (i.e., pH, enzymes, chemical receptors) unique to mosquito larvae must be present to result in toxicity. Bs has been shown to be effective against many mosquito genera. All species of *Culex* larvae are considered susceptible to Bs, and many species of *Aedes*, *Psorophora*, *Coquillettidia*, *Mansonia* and *Anopheles* are also very susceptible. However, susceptibility of species within these genera is variable. Studies of Bs clearly indicate that it is not infectious or pathogenic.

USEPA does not require formal environmental fate data for Bs given its nontoxic nature to non-target organisms. The length of time that Bs remains effective against mosquitoes varies, depending primarily on the species and behavior of mosquito larvae, environmental conditions, and water quality. In particular, Bs appears to recycle in the cadavers of dead mosquito larvae. This means that, in general, the more larvae that are killed in the initial application, the longer the residual action. In general, Bs is effective for one to four weeks after application, although measures of effectiveness range from as little as 2.5 hours to more than 60 days. UV light in the range of 300 to 400 nm, falling within the wavelength range of sunlight, has been shown to inactivate both spores and exotoxins of Bs. Bs is less likely than Bti to adsorb to particulate matter and settle out of the water column. Therefore, it is considered to have generally higher efficacy against mosquito larvae in waters with a higher degree of particulates. As it occurs naturally, Bs does cycle and maintain itself in the environment; however, the insecticidal

formulations currently in use do not cycle in salt water to infect subsequent generations of mosquito larvae (but will in fresh water). Bs is relatively slow acting, compared to Bti. Larvae in a treated area may hatch, and develop through the first two larval stages prior to being controlled. For this reason, once an area has been treated, it should not be re-treated unless stages III and IV are present. SCVC field crews have been specially trained to understand this effect in order to avoid unnecessary re-treatments.

Bs is generally not considered a risk for non-target organisms. The commercially available form of *Bs*, VectoLex, has been extensively tested and is considered non-toxic to non-target organisms. USEPA concluded that Bs does not pose any significant risk to non-target organisms or the environment. Bs has been used by SCVC since 1997. It is particularly favored by the field crews, who have observed firsthand that the sustained action of this material saves them work by requiring fewer re-treatments.

Methoprene

Methoprene is a biochemical pesticide found in two formulations (methoprene and methoprene sustained release formula) and is registered under the Altosid trade name line. Methoprene is used to control mosquitoes, beetles, horn flies, tobacco moths, sciarid flies, fleas (eggs and larvae), fire ants, pharaoh ants, midge flies, and Indian meal moths. It is also registered for use on a number of foods including meat, milk, eggs, mushrooms, peanuts, rice, and cereals. There are also uses in food processing plants and eating establishments; along with non-food uses such as for tobacco, ornamentals, golf courses, pet products, uses in and around the home, and in boxcars.

Methoprene is an insect growth regulator that acts by interfering with maturation and reproduction in insects by mimicking the activity of natural juvenile insect hormone. This hormone in insects, secreted by glands near the brain, controls the retention of juvenile characteristics in larval stages. If present, it (or methoprene acting as an insect growth regulator) leads to a suppression of adult characteristics. Methoprene may also be toxic to non-insects through similar effects. Although applied at the larval stage, response to methoprene usually occurs in the last instars of the larval or nymph form, or pupae form. In the case of mosquitoes, larvae are the target stage, but the effect is not seen until lack of adult emergence.

Methoprene degrades rapidly in sunlight, both in water and on inert surfaces. Within three days of application, 90 percent will degrade via photolysis and microbial metabolism; without microbial metabolism, photolysis will degrade 80 percent in 13 days. Overall, methoprene has a half-life ranging from 30 hours to 14 days, depending on environmental conditions (some higher half-lifes have been reported for non-aqueous environments). Higher temperatures and salinity lead to higher degradation rates. The effects of methoprene last up to a week, but it reaches undetectable levels in ponds within 48 hours of application. After four days, only one percent of the original application concentration will persist in the top two inches of soil. Methoprene is tightly adsorbed to soil and is rapidly broken down; therefore it is not likely to be transported to ground water. Methoprene sustained release formulation does not produce residual concentrations greater than those produced with the application of the liquid formulation. Sediment sampling associated with the Caged Fish experiment suggested that methoprene has a half-life in aquatic sediments of less than one week.

Methoprene is generally considered to be slightly to non-toxic to terrestrial wildlife. Methoprene is considered slightly toxic to birds. Methoprene may have some impact on honeybee foraging, although definitive data are pending. When methoprene is used as a mosquito larvicide, honeybees are unlikely to be exposed to the product, as it is applied so as to affect mosquito larvae in aquatic settings, and not bees concentrating on collecting pollen.

Methoprene is considered moderately toxic to warm water, freshwater fish, and is slightly toxic to cold water, freshwater fish. Methoprene is considered highly toxic to freshwater invertebrates. For amphibians, mortality has not been observed at concentrations up to 1.3 ppm (leopard frog). However, adverse effects such as reduced body weight and developmental delays at 720 ppb (leopard frog) were observed. In recent years, methoprene has received considerable attention as a possible causative agent of the increase in amphibian malformations. The theory that methoprene might mimic the action of retinoids and cause malformations in amphibian populations is partially supported by research on how methoprenic acid (t-MA) can stimulate gene transcription in vertebrates, particularly amphibians during metamorphosis. Much of this theory, however, remains largely unsupported by ancillary information and anecdotal reports, as well as contradictory findings within and outside of the taxon. Research in this area is considered ongoing and future experimental findings and other developments warrant attention.

However, measurements of methoprene concentrations made during the Caged Fish experiment found that all measurements (except those immediately after applications) were less than 1 ppb, and all environmental measurements of mosquito control applications in Suffolk County have been at least two orders of magnitude below the 720 ppb threshold reported for the leopard frog impacts.

At sufficient concentrations, methoprene can be very highly acutely toxic to estuarine and marine invertebrates, as seen in studies with grass shrimp and mud-crabs. However, this toxicity was observed at levels far greater than those that result from mosquito larviciding. Marine organisms are not likely to be exposed to methoprene, but estuarine organisms are likely to be exposed as a result of application within estuarine habitats. Methoprene degrades rapidly in water so the use of most formulations in estuaries is generally not of concern. However, concern has in fact been raised in recent years with respect to methoprene's potential impact on shrimp, crabs and lobsters. These concerns stem from the fact that a shared evolutionary past, as well as resultant similarities in biology, exist between crustaceans and dipteran species (including mosquitoes). These concerns were heightened by events such as the widely-publicized 1999 Long Island lobster die-off, although subsequent studies have indicated environmental and not chemical causes. Most of the recent studies of estuarine invertebrates have used shrimp, Atlantic oysters, amphipods, copepods, and mud crab. In general, impacts to these species are not anticipated to occur at expected environmental concentrations. The Caged Fish experiment found no impact to exposed fish and shrimp, for example. The risk analysis found no risks for impact to ecosystems because exposures were much less than the levels required to cause impacts to organisms. The lowest concentrations found to cause impacts to lobsters, when the exposures were sustained for days, were only recorded immediately after applications in local sampling. This reinforces the conceptual understanding that the effectiveness of methoprene on mosquito larvae occurs at concentrations below those necessary to cause significant non-target organisms effects. Methoprene has been used by SCVC since 1995, and is particularly useful in the salt marsh, where Bti is not always effective.

The summary of the selected larvicides potential impacts (as determined through the quantitative risk assessment) are included in Tables 16 and Table 17.

Table 16. Summary of the Human Health Risk Assessment for Larvicides

Agents Considered	Most Critical Endpoint Considered	Pathway Considered Potential Risk	Locations with Potential Risk	Conclusion in Risk Assessment	Comments	Role in Management Plan
Methoprene	NA	Not expected to be human health risk due to limited exposure	No locations were of concern	Not expected to be human health risk	Not quantitatively evaluated because exposure expected to be minimal	Preferred larvicide based on effectiveness for all larvae Stages, used in combination with Bti
Bti	NA	Not expected to be human health risk due to limited exposure	No locations were of concern	Not expected to be human health risk	Not quantitatively evaluated because exposure expected to be minimal	Preferred larvicide effective for Stage I, II & III larvae
Bs	NA	Not expected to be human health risk due to limited exposure	No locations were of concern	Not expected to be human health risk	Not quantitatively evaluated because exposure expected to be minimal	Preferred larvicide effective for Stage I, II & III larvae. Especially good in polluted, freshwater habitats used by Culex spp.

Table 17. Summary of Ecological Risk Assessment for Larvicides

Agents Considered	Terrestrial Birds, Mammals, Reptiles	Terrestrial Insects	Aquatic Life	Comments	Conclusion in Risk Assessment	Role in Management Plan
Methoprene	No risk*	Not expected to be terrestrial risk due to limited exposure	No risks*	Not quantitatively evaluated because exposure expected to be minimal	No ecological risks*	Preferred larvicide based on effectiveness for all larvae Stages, used in combination with Bti
Bti	No risk*	Not expected to be terrestrial risk due to limited exposure	No risks*	Not quantitatively evaluated because exposure expected to be minimal	No ecological risks*	Preferred larvicide effective for Stage I, II & III larvae
Bs	No risk*	Not expected to be terrestrial risk due to limited exposure	No risks*	Not quantitatively evaluated because exposure expected to be minimal	No ecological risks*	Preferred larvicide effective for Stage I, II & III larvae. Especially good in polluted, freshwater habitats used by Culex spp.

* That is, predicted exposures were below levels of concern established by USEPA and/or others and so do not indicate that there is an increased risk of unacceptable ecological impacts from use of the pesticides under the conditions evaluated in this assessment

6.6 Formulations and Uses

There are five basic Bti formulations available for use: liquids, powders, granules, pellets, and briquets. Liquids, produced directly from concentrated fermentation slurry, tend to have uniformly small (two to 10 micron) particle sizes, which are suitable for ingestion by mosquito larvae. Powders, in contrast to liquids, may not always have a uniformly small particle size. Clumping, which results in larger sizes and heavier weights, can cause particles to settle out of the feeding zone of some target mosquito larvae, preventing their ingestion by the typical filter feeding process used by these insects. Powders must be tank-mixed before application to an inert carrier or to the larval habitat. They must be mixed thoroughly to achieve a uniformly small consistency. Bti granules, pellets, and briquets are formulated from Bti primary powders

and an inert carrier. Bti labels contain the signal word "CAUTION" (see Appendix 2). SCVC will predominantly use liquid and briquette formulations.

Available commercial brands of Bti liquids include Aquabac XT, Teknar HP-D, and Vectobac 12AS. Labels for all three products recommend using four to 16 liquid oz. per acre in unpolluted, low-organic water with low populations of early instar larvae (clean water situations). The Aquabac XT and Vectobac 12AS (but not Teknar HP-D) labels also recommend increasing the range from 16 to 32 liquid oz. per acre when late third or early fourth instar larvae predominate, larval populations are high, water is heavily polluted, or algae are abundant. Bti liquids will be applied by air or truck, with or without methoprene in a duplex formulation.

Bti briquets (donuts) are a mixture of Bti, additives, and cork. They are designed to float and slowly release Bti particles to the water body for extended periods of time. They apparently are attractive to raccoons because of their odor, and may sometimes be disturbed or carried off (other wildlife may also feed on them). Donuts may be staked in place to prevent wind from moving them from a site's littoral zone into open water. The use rate is one donut per 100 square feet in clean water and up to four donuts per 100 square feet in water with high particulate levels ("dirty water"). They are available for use in recharge basins, pools, and, potentially, catch basins, although the difficulties associated with highly organic water make these somewhat less preferred than either Bs or methoprene.

Corncob granules use a carrier that is dense enough to penetrate heavy vegetation. There are currently two popular corncob granule sizes used in commercial formulations. Aquabac 200G, Bactimos G, and Vectobac G are made with 5/8 mesh size grit-crushed cob, while Aquabac 200 CG (Custom Granules) and Vectobac CG are made with 10/14 mesh size grit cob. Aquabac 200 CG is available by special request. The 5/8 mesh size grit is much larger and contains fewer granules per pound. The current labels of all Bti granules recommend using 2.5 to 10 lbs. per ac. in clean water and 10 to 20 lbs. per ac. in dirty water situations. SCVC uses these products to larvicide on Fishers Island.

VectoLex-CG is the trade name for a granular formulation of Bs (strain 2362). The product is formulated on a 10/14 mesh size ground corncob carrier. The VectoLex-CG label carries the "CAUTION" hazard classification. Bs is designed to be applied by ground (by hand or truck-

mounted blower) or aerially at rates of five to 10 lbs. per ac. Use of the highest rate is recommended for dense larval populations. VectoLex WSP, a water-soluble pouch, is registered for use in catch basins, and is a recommended product for them. They are also used in fresh water habitats that hold their water, because the cycling of the bacteria provides additional control over time. Bs is not suitable for habitats that dry down, as the bacteria will perish.

Altosid is the name of the methoprene product used in mosquito control and is applied as briquets (similar in form to charcoal briquets), pellets, sand granules, and liquids. The Altosid label carries the "CAUTION" hazard classification. The liquid and pelletized formulations can be applied by helicopter and fixed-wing aircraft.

Altosid Liquid Larvicide (A.L.L.) and A.L.L. Concentrate**:** These two flowable formulations have identical components except for the difference in the concentration of active ingredients. A.L.L. contains five percent (wt./wt.) s-methoprene while A.L.L. Concentrate contains 20 percent (wt./wt.) s-methoprene. The balance consists of inert ingredients that encapsulate the s-methoprene, causing its slow release and retarding its ultraviolet light degradation. Use rates are three to four oz. of A.L.L. five percent and 0.75 to one ounce of A.L.L. Concentrate (both equivalent to 0.01008 to 0.01344 lb. AI) per ac., mixed in water as a carrier and dispensed by spraying with conventional ground and aerial equipment. A.L.L. Concentrate is recommended for aerial and truck applications.

The Altosid Briquet was the first solid methoprene product marketed for mosquito control beginning in 1978. It is made of plaster (calcium sulfate), 3.85 percent (wt./wt.) r-methoprene, 3.85 percent s-methoprene (0.000458 lb. AI/briquet) and charcoal to retard ultraviolet light degradation. Altosid Briquets release methoprene for about 30 days under normal weather conditions. Application should be made at the beginning of the mosquito season and under normal weather conditions repeat treatments should be carried out at 30-day intervals. The recommended application rate is one briquet per 100 square feet in non-flowing or low-flowing water up to two feet deep. Recommended treatment sites include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, abandoned swimming pools, construction sites, and other artificial depressions. Altosid also comes as a XR Briquet**,** made of hard dental plaster (calcium sulfate), 1.8 percent (wt./wt.) s-methoprene (0.00145 lb. AI/briquet) and charcoal to retard ultraviolet light degradation. Despite containing only three times the AI as the

"30-day briquet," the comparatively harder plaster and larger size of the XR Briquet change the erosion rate allowing sustained s-methoprene release up to 150 days in normal weather. Altosid Pellets were approved for use in April of 1990. They contain four percent (wt./wt.) s-methoprene (0.04 lb. AI/lb.), dental plaster (calcium sulfate), and charcoal. As with the briquets discussed above, Altosid Pellets are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 30 days. Label application rates range from 2.5 lbs. to 10.0 lbs. per ac. (0.1 to 0.4 lb. AI/ac.), depending on the target species and/or habitat. This formulation is effective in penetrating habitats with overhanging vegetation. It is also suitable for wetting-drying habitats, as not all of the product dissolves at once, and so it can provide residual impacts when the habitat wet again.

Storm water structures should receive either Vectolex WSP pouches or Altosid briquets as a preferred treatment. If the recharge basin being treated appears to have clear water, treatment with Bti donuts is possible, and may indeed be preferred due to the general difficulty of inducing resistance with Bti.

Field crews will have equipment that allows treatment of any site with Bti, Bs, or methoprene. Treatment will depend on the combination of the stage(s) of the larvae, and environmental conditions. Vectolex may be preferred in swampy situations, as it has greater penetration through undergrowth due to the weight of the pellets. The crew leader is responsible for carefully estimating the area of the application (based on dimensions of the application, so that 100 feet by 100 feet is one-quarter of an acre, for example), and determining the amount of product to be used. In-house and NYSDEC pesticide applicator training enable these calculations to be made in a manner consistent with the law and the appropriate label.

Aerial application decisions will be made based on surveillance data. As stated earlier, Bti is often used for early season applications, and methoprene is often the choice for middle of the summer. Applications should be made at very low altitudes to minimize drift.

Table 18. Larvicide Decision Table

Surveillance Means	Result Class	Quantitative?	Resultant Action
Aerially-larvicided salt marsh	Presence Area Present Stage	@ Wertheim NWR Potentially expandable	Stages I- II: Bti Older: methoprene
Other salt marshes	Presence Stage	No	Stages I- II: Bti Older: methoprene
Permanent Fresh Water Habitat	Presence Stage Environmental Considerations	Possible	Stages I- III: Bs Older: methoprene
Transient Fresh Water Habitat	Presence Stage Environmental Considerations	No	Stages I- III: Bti Older: methoprene
Catch Basins	Presence	No	methoprene time release
Recharge Basins	Presence Environmental Considerations	No	Stock fish Transient: Bti donuts Permanent: Bs Methoprene time release
Artificial (e.g., swimming pools)	Presence	No	Empty If not possible: Bti, methoprene

6.7 Efficacy Measurements

As part of the reorganization of SCVC, a "QA-QC" team should be developed. The intent of this team is to measure the effectiveness of actions taken by SCVC to control mosquitoes. It is clear that the use of pesticides by SCVC is one of the more important elements of the measure of effectiveness of the program. Therefore, a major effort should include the testing of the effectiveness of larvicide applications.

The three major larvicide efforts could be included:

- Catch basins
- Non-aerial larvicide applications (routine monitoring responses, and complaint follow-up)
- Aerial applications

The QA/QC team will have access to application data so that testing is appropriate to the treatment.

Catch basin work is not time sensitive. An appropriate scale of work might be follow-up at a rate of 20 basins per month (tentatively, five basins in four general treatment areas) to dip for larvae to ensure:

1) Untreated basins are not now breeding mosquitoes

2) Treated basins are not now breeding mosquitoes

The intent of the work is to guide the future actions of the field crew to enhance efficiency and ensure that effective treatment is occurring.

Non-aerial larvicide application testing is time sensitive. These sites will need to be visited within a day or two of treatment to sample in a fashion appropriate to treatment. If Bti or Bs were applied, then dipping for larvae is the appropriate measure of success. Bti should kill larvae within 24 hours and so finding live larvae signals that the treatment was not completely successful. For Bs, the finding of stage I and II larvae does not indicate that this slow acting material is not working. Only the presence of stages III, IV and/or pupae indicate that a Bs treatment is no longer working. This test will need to be a relative measure, however, as it is not clear that quantitative determinations of larval density can be based on dip sampling. Discussion with field crews if live larvae are found to determine pre-treatment relative larval densities may assist in deciding if the treatment was successful or not. If methoprene was applied, or a duplex treatment was made, larvae or pupae should be sought for "fly-up" testing. The organisms can be brought back to the laboratory, and their development history traced. Failure to develop is a signal that the pesticide application was successful, although transfer to the laboratory sometimes results in failure to thrive.

In either situation, the measurements will be more effective if similar, untreated wetlands are sampled concurrently to act as control sites. Again, because of the nature of the sampling methodology, it is unclear if the results can always be quantitatively compared.

A similar procedure should be followed to assay the effectiveness of aerial larviciding.

It needs to be understood that tidal flushing in salt marshes, and access issues in fresh water wetlands, will complicate these efforts. The best site selection would be to use a random

selection process, but this must be tempered by practical considerations, to avoid unnecessary travel over a large County to merely satisfy random selection criteria. It should be understood that a geographically appropriate selection of sites would be best, made over the course of the season, and that a good selection of the kinds of treatments made by staff would also be appropriate.

The frequency of testing would be biased towards non-aerial applications, as numerically they represent the majority to applications made by SCVC. Optimal frequencies may be best determined once the program is established; as a coarse estimate, something in the vicinity of 20 sites for truck applications of larvicides, and two aerially larvicided marshes tested each month through the season, seems to be a minimal effort required to develop efficacy information.

The existing New Jersey trap network comprises a programmatic measurement of the effectiveness of source reduction and larviciding. That is, they not only measure whether larvicidal treatments are working, they measure whether enough sites are being treated to achieve an effective result. From a programmatic perspective, it is not enough that for larval control methods to be effective, they must also be used in enough mosquito sources to provide area-wide control. This is especially true for salt marsh species, where only a few uncontrolled acres can infest hundreds of acres of residences after a major hatch. Measurements of adult mosquito population changes at set sites, over longer periods of time so that immediate climatological impacts or individual year's variations are not a factor, will clearly show if the program has been able to reduce the generation of biting adults. The ABDL has conducted such a study, using seven years data prior to the introduction of methoprene, and seven years after its introduction. It is clear from the trends that methoprene resulted in almost an order of magnitude reduction in overall adult mosquito counts, at nearly all of the salt marsh sited traps.

New Jersey trap data are relatively blunt instruments, however. The traps tend to collect mosquitoes from an area and it may not possible to link specific marshes to specific trap counts. Similarly, reductions in larval survival at one marsh may be balanced by increased breeding at a second, uncontrolled site, which means the data will not reflect the effectiveness of treatment at the first marsh.

The use of a specialized QA/QC team in the program would mean that measurements of efficacy will not be compromised by resource competition. It is very important that SCVC be able to provide information to the public that justifies the actions it has undertaken, and that this information clearly identifies treatments that were effective – and those that may not have been as effective.

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7. Control of Adult Mosquitoes

7.1 Background Information

Treatment of adult mosquitoes is the control practice most linked by the public to vector control agency operations, although it actually is the last option in terms of effective control possibilities. Most often, this treatment is with pesticides, known as adulticides because they kill adult mosquitoes. Adulticides can be applied either by ground or by air, most commonly via ultra low volume (ULV) or thermal fogging techniques.

Control of adult mosquitoes is generally viewed as the last line of defense against these vectors. It may be the least efficient, as well, because adult mosquitoes are generally dispersed, and are associated with a medium, air, where control chemicals are difficult to concentrate so as to achieve the greatest effect. The term "adulticiding" is used to describe applying insecticide to eliminate adult mosquitoes, either while the insects are flying or resting in vegetation, in (or on) buildings, or in other sites of harborage. These applications can be made from the ground, via truck-, cart-, or backpack-mounted machines, or the air, via airplanes or helicopters, and are mostly applied using ULV equipment. ULV is the application of small amounts of highly concentrated insecticide. The actual amount of insecticide applied is typically in the range of 0.00117 to 0.076 pounds of active ingredient per acre, depending on the insecticide used. This very low application rate is intended to minimize human health and non-target impacts, but these low concentrations are effective at killing mosquitoes (the effective concentrations are much less than those used against hardier agricultural pests, for example). The insecticide is applied using application equipment that produces small droplets that remain airborne and are designed to contact flying mosquitoes. For ULV applications, the droplet size produced is generally in the 10 to 50 micron size range, depending on the chemical used and the specific label application recommendations.

Older mosquito spraying technology depended on "thermal fogging," which aerosolized a petroleum/insecticide mix, creating a thick white fog. Thermal fogging is still considered useful under certain conditions, especially when penetration of relatively enclosed places is required. It is considered essential for the treatment of tire piles, for example. Mosquito control districts that treat underground storm water systems to control adult mosquitoes also typically use thermal

fogging. ULV is generally preferred, because such treatments use less insecticide per acre. This results in:

- smaller environmental exposures
- savings in insecticide costs
- reductions in the use of diluents (minimizing potential environmental and human health impacts from these compounds, and also reducing costs)
- more efficient loading and transporting pesticides.

Another advantage of ULV spraying is avoidance of dense fogs, which are produced by thermal fogging, and can cause problems due to reduced visibility (such as traffic hazards).

There has been considerable evolution in pesticides used for adulticiding. At one time, DDT and other chlorinated hydrocarbons were used. Then, for decades, organophosphates were used almost exclusively. Currently, most mosquito control agencies, including Suffolk County, rely on synthetic pyrethroids, which are chemically similar to naturally occurring pyrethrins. Pyrethroids, in general, do not have large impacts on the environment and are reported to have extremely low mammalian toxicity. However, the widespread use of pyrethroids should not be construed to mean that organophosphates, such as malathion, are not useful. Malathion can be very important if pyrethroid resistance develops in the local mosquito population, for example.

Adulticides used in the US include the organophosphates malathion, fenthion, naled, and chlorpyrifos, and the pyrethrins and pyrethroids. Natural pyrethrins (pyrethrum) are extracted from chrysanthemum flower heads. Pyrethroids are synthetic analogues of the natural pyrethrins, and include resmethrin, sumithrin, and permethrin.

Technical factors that need to be managed for adulticide applications include identifying the target mosquito species, setting the droplet size and dosage rate, and understanding the environmental conditions that will affect the delivery of the pesticide. Delivery systems must be calibrated and managed so as to apply the right dosage to achieve maximal mosquito control and minimal unintended impacts. Suffolk County has recently purchased a model through the

resources of the Long-Term Plan development project to help insure optimal application of the selected pesticides (the Adapco Wingman system).

The decision to apply adulticides must be based on information drawn from scientifically-based surveillance activities. Having stated that, the decision will not be based on a single treatment threshold. Applying an adulticide to control mosquitoes is a decision based on the mosquito species, the numbers of mosquitoes present, the threat or presence of a human pathogen, the age and history of the mosquito population of concern, and the time of year. In addition, historical and current trends in the mosquito populations, the current weather, the predicted weather, both short-range and over an extended period of time (seasonality), the environmental setting, and the people in the area where the pesticide will be applied also need to be factored into this equation (see Table 19). These various factors form a risk determination by program managers, where potential benefits (and potential costs) of applying the pesticide are weighed against the probable costs (and potential benefits) of not applying the pesticide. The costs of not applying the pesticide are the only element described as probable, because at the time of application the present impact of the mosquito population to human health and public welfare is the most well-known factor under consideration. In addition to this complex set of variables, there is also, to a certain degree, the expressed preference of the community that may or may not receive the treatment. Nonetheless, when vector control treatments are being considered, one necessity for an application will be measurements of 25 or more female mosquitoes of human-biting species per trap night when New Jersey traps are used, and, with CDC light traps, 100 or more female mosquitoes from human-biting species. A vector control application in the absence of any trap data can only occur under extremely extraordinary conditions.

Table 19. Adulticide Decision Parameters

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Basic Surveillance Parameters	Number of mosquitoes	Yes	No	Counts in light traps significantly above criteria; landing rates; complaints	Based on female, human-biting species; 25+ per NJ trap, 100+ per CDC trap; landing rate 1+/min.; complaints invaluable where traps are not set; intend to set CDC traps before all non-Fire Island applications
	Species present	Yes	Yes	Light trap content analysis	Information on basic mosquito biology essential: Vector Control targets aggressive biters; Health Emergency targets specific (bridge) vectors; ; intend to set CDC traps before all non-Fire Island applications
	Complaints	Yes	Yes	Number/location of calls	Evaluate in historic context; complaints must be supported with appropriate surveillance data; complaints document extent of problem better than traps can
	Historical population trends	Yes	No	Surveillance data records	Data patterns often signal that problem is about to abate, or is likely to worsen
Species Specific Parameters	Aggressiveness of target species	Yes	Yes	Documented biting patterns of trapped mosquitoes	Aggressive biters indicate greater problem, increased likelihood for bridge vector participation
	Activity patterns of target species	Yes	Yes	Documented host seeking patterns, flight ranges of trapped mosquitoes	Guides actual control decision; e.g., evening vs. later at night; day-time flying may inhibit control; spot treatments only effective for short flight range species; large flight ranges require applications to cover larger, continuous areas to be effective
	Vector Potential	No	Yes	Infection rate, vector competence, % mammalian meals of trapped species	Establishes relative risk for species present
	CDC Vector Index	No	Maybe	MIR, trap counts for all potential vectors	CDC light trap counts * MIR, summed over all vector species; higher index correlates to more human infections following week; requires high mosquito/human infection rates for use; can use only with multiple trap data sets

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Species specific parameters, continued	Parity rates	Sometimes	Yes	Age (blood meal history) of biting population	For Health Emergency, high parity rates indicate majority of biters had prior blood meal – direct indication of increased Vector Potential; for Vector Control, an aging population, even if smaller, will be treated since it represents increasing vector potential
	Life Cycle Type	Yes	Yes	Trap analysis	Brooded mosquitoes eventually die off on own, continuous breeders build populations over season
Public Health Parameters	Bird testing	No	Yes	Presence/absence of virus	Provides early warning in terms of bird to bird transmission; documents active disease foci in County
	CDC mosquito pool testing	No	Yes	Presence/absence of virus	Amplification vectors provide early warning, document active disease foci in County; bridge vectors indicate virus present in human-biting species, is signal that human health risk is imminent
	Veterinarian reports	No	Yes	Ill/dead target animals	Non-mammals provide early warning, document active disease foci in County; mammalian cases indicate virus present in bridge vectors, signal that human health risk is imminent
	Physician reports	No	Yes	Human cases	Realized human health threat
	Disease history	No	Yes	Number of human/ important animal cases in prior years	Indicates that local conditions are favorable for pathogen amplification and transmission
	Avian dispersal/migration patterns	No	Yes	Time of year regarding dispersal of hatch year birds and known migration periods	Identifies new areas for concern, signals need to control known bridge vectors
Climatic Parameters	Current weather	Yes	Yes	Temp = 65+ Wind < 10 mph No rain	Application time decision
	Short-term weather forecast	Yes	Yes	Presence of fronts & storms; barometric patterns	Application planning
	Time of year	Yes	Yes	Spring, Summer, & Fall activity patterns for trapped mosquitoes	Species-specific behavior; generally, cooler weather retards activity, warmer weather increases activity; virus presence not as significant when activity decreases

Type of Parameter		**Factor for Vector Control Applications?**	**Factor for Applications under Health Emergency?**	**Criteria**	**Comment**
Ecological Parameters	Environmental factors in target area	Yes	No	Environmentally sensitive settings (R-T-E species)	Prior mapping is essential to clearly identify all environmentally sensitive areas; usually addressed through NYSDEC; Town and other expert cooperation is sought
	Population	Yes	Maybe	Number of impacted people/population density	For Vector Control: no people means no problem; for Health Emergency, threat may be sufficient
	Application restrictions	Yes	In some settings	Farms; no-spray list; NYSDEC wetlands, wetlands buffers; open water buffers	Vector Control no-spray areas include crop areas, no-spray list, buffers – discontinuities may make application ineffective

The control of adult mosquitoes means managing their populations so that they cause less of an impact to people. Suffolk County has a pesticide phase out law that sets a goal of limiting or eliminating pesticide use when possible. Mosquito adulticides must be used in residential areas to control mosquitoes that are biting people. This means that human exposure to the materials is inevitable, and efforts to minimize exposure to pesticides are prudent. In addition, it is at least theoretically possible that there are as yet unknown adverse impacts that could result from use of these materials, so that it is wise to place limits on their use.

It is anticipated that more effective control of mosquitoes of concern, through source reduction (especially progressive water management project implementation) and also through more effective larval control driven by enhanced surveillance, will result in less adulticide use. Optimization of adulticide delivery through the Adapco air guidance system (see below) will also reduce insecticide use.

The interest in reducing or eliminating pesticide use is expressed in the evaluations of alternatives to pesticides. This section will begin by discussing some of the most commonly discussed alternatives to adulticides, and evaluate their potential for ensuring protection of human health and the alleviation of discomfort that often accompanies large or dangerous populations of adult mosquitoes.

7.2 Alternatives to Adulticides

There are four general alternatives to adulticides:

- Use of personal protection/avoidance of mosquito conditions
- Barrier treatments
- Enhanced predation
- Traps

Personal protection steps

Mosquitoes may be avoided. Steps can be taken, including the installation of screens; in some areas, mosquito nets (especially those impregnated with pesticides) are commonly used. Widespread use of air conditioning tremendously reduces contact with mosquitoes, as some mosquitoes can negotiate screens. This can mean isolating oneself from the portion of the world the mosquito inhabits, and avoiding outside activities during the part of the year that for many is when outside activities are most common (i.e., summer).

It is possible to find effective means of repelling mosquito bites. The compound DEET (N,N-diethyl-m-toluamide) was first registered as an insect repellent in 1957. It is used to repel biting insects, such as mosquitoes, ticks and flies. It is believed that DEET repels insects by interfering with the insect's ability to sense or locate animals to feed on. DEET can be used in homes, applied directly on the skin and clothing, and can be used to protect animals, such as dogs, cats and horses. The percentage of DEET in products can vary, ranging from about five to 100 percent. It is remarkably effective. Studies of DEET have shown consistent abilities to allow people to share space with mosquitoes seeking blood meals and yet avoid nearly all bites.

Up to 20 percent of a dermal application of DEET can be absorbed through the skin. It is generally eliminated through urine within several hours, and does not accumulate. Use of sunscreens with added DEET may enhance absorption.

There have been some reports of seizures in children using DEET products. The number of cases of effects appears to be quite small, given broad estimates of 50 to 100 million users each year. USEPA concluded that although DEET was implicated in certain seizure cases, evidence of it causing the seizures was insufficient to directly link DEET use to this health impact. Nonetheless, USEPA suggested it is prudent to exercise caution in the use of DEET directly on the skin. There are some indications that long-term use may have some negative effects, although these reports are either from animal studies or are anecdotal. Studies of synergistic effects of DEET with other chemicals (from Gulf War Syndrome research) are not conclusive (see the discussion of permethrin, below, for more details).

The US Army has found it difficult to ensure that soldiers use DEET as ordered. Compliance rates, even when under orders, are as low as 50 percent. Aesthetic problems, including the feel of the repellent on the skin and its odor, are cited (as well as fears associated with some of the health concerns raised above). The Army is now developing its own alternative to DEET.

Some repellents are said to be "just as good" as DEET. Most do not measure up in independent research. Some that have fared well include:

- Picaridin (a European product) recently received approval as effective by the CDC and registered in New York State
- BiteBlocker (a botanical product)
- Oil of eucalyptus (a botanical product) recently received approval by CDC as an effective repellent

Citronella based products has not measured up, despite word of mouth to the contrary. It may be that reactions between an individual's skin/skin chemicals/other applied soaps, perfumes, etc., result in particular combinations that serve to repel mosquitoes. This may account for products that have fierce loyalties, but test poorly. However, for citronella, Health Canada has raised concerns regarding potential negative impacts to people from use of the material on the skin.

All-in-all, it is clearly possible for individuals to construct effective means of fending off mosquitoes through personal actions. However, there may be some negative health or social impacts associated with mosquito avoidance. Also, note that NYSDOH guidance for when people face large numbers of biting mosquitoes is to use DEET on exposed skin, with permethrin-impregnated clothing as an additional repellent measure. This increases the individual's exposure to pesticides. The US Army also relies on permethrin-soaked clothing. The use of permethrin-soaked clothing as a mosquito deterrent may cause exposures to pesticides that are much higher than would occur generally in Suffolk County under its proposed IPM program, and than would occur even in areas with highest adulticide usage. Nonetheless, if significant portions of the population were persuaded to stay indoors more, and to use effective repellents when outdoors, the need to adulticide would be less. However, if a significant

proportion of the population did not comply, public health officials would very likely still determine that the risk equation favored the use of adulticides to avoid human illness.

Barrier treatments

These products primarily function as area versions of the human repellents listed above. The idea is that mosquitoes will avoid the area where they have been applied. Mostly, they are considered minimum risk compounds by USEPA and therefore do not require federal registration under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Further, in most states, these products are not classified for regulatory purposes as pesticides, so posting, notification, and reporting laws do not apply. This means that the information available on these products is less than for products with more regulation. Please note that even minimum risk compounds undergo regulatory scrutiny in New York State.

Most efficacy information is not from published articles in scientific or professional journals, but rather sponsored reports or testimonials. Products typical of the class include:

- EcoSmart Technologies Corporation developed EcoExempt IC, an insecticide concentrate containing a blend of plant oils, labeled for outdoor yard and barrier treatment for mosquitoes. The only available data on this product comes from company sponsored tests, reporting immediate knock-down of flying mosquitoes in laboratory settings. No non-target impacts or field studies are available. The chemicals in the product can sometimes damage its application equipment.

- Garlic and garlic oils have been touted as mosquito repellents, and, sometimes, pesticides. Two well-known garlic-based adult mosquito repellents the Mosquito Barrier (or the Garlic Barrier), and Mosquito and Gnat Scat. Mosquito Barrier reportedly kills adult mosquitoes and repels them from treated areas, and, when mixed with canola oil, will kill mosquito larvae. SCVC has performed local tests on garlic oils as barrier treatments informally, and they were tested as part of the Long-Term Plan Early Action Projects. The tests showed that garlic oils could reduce the number of mosquitoes reaching mosquito traps in treated areas by up to 50 percent, as compared to non-treatment areas.

Mosquito Scat is an herbal oil preparation containing three oils as active ingredients: lemon grass oil, peppermint oil, and garlic oil. It is sprinkled outside to repel mosquitoes. One university study found no significant differences in mosquito numbers in the comparison of treated versus untreated areas.

Generally, garlic repellents do not appear to have worked well at discouraging mosquitoes, especially when densities were higher. It can be argued that a 50 percent reduction in numbers, which were the best results achieved with local testing, is not sufficient to eliminate a "problem." This is especially so when mosquito counts are high. For instance, New Jersey light trap data used by SCVC generally finds background, i.e., "non-problem," quantities of aggressive mosquitoes to be in the vicinity of five per night. By most definitions, a count of 25 aggressive mosquitoes per night constitutes a biting problem. Reducing a count of 25 by 50 percent reduces the impact, but does not return conditions to background levels. Reducing the count by 80 percent or more would reduce the count to background levels. Therefore, using this simple example, minimum efficacy necessary to "treat" a mosquito problem is on the order of 80 percent.

In addition, the garlic oil products were notably not effective against *Cx. pipiens* in upland settings, and did not repel *Oc. sollicitans* very well in coastal settings. Since these are the two mosquitoes of greatest management concerns in each particular setting, it again suggests that use of the product to achieve management aims is not advisable.

- Hand-held electronic devices relying on high-frequency sound to repel mosquitoes continue to come to the marketplace. They often claim to work by mimicking the wing beat frequency of a male mosquito or even the wing beat frequency of a hungry dragonfly. Scientific studies have repeatedly shown that electronic mosquito repellers do not prevent host-seeking mosquitoes from biting.

- The Mosquito Cognito emits a chemical that, according to the manufacturer, has a unique scent-blocking ability, because it binds to mosquito olfactory receptors and blocks the mosquito's ability to smell people and animals. The Mosquito Cognito looks something like a square suitcase or ice chest, and can be placed outside on decks or porches where people are gathered. A university report found

While it can keep distant mosquitoes from locating people, it cannot prevent nearby mosquitoes from locating people by vision or by the person's thermal emissions. For this reason, it is inappropriate for use within mosquito habitat.

- Mosquito Barrier, a citronella and rosemary solution, was applied by the Long-Term Plan to several sites. This product was touted as an effective mosquito repellent and is similar to Mosquito Scat, albeit with no garlic in it. The product did not prevent mosquitoes from reaching mosquito traps in the middle of treated areas at the same rate they reached traps where no treatments were made.

None of these products has demonstrated the level of efficacy offered by pesticides, which generally are found to reduce mosquito counts by 90 percent. In addition, these products tend to be limited in the aerial coverage they offer, and may merely deflect mosquitoes to another site. This means that, as a relatively rare use, any one of the products could be effective and provide some degree of comfort for those people in a specific area affected by the product. However, it is far from clear that the products would maintain effectiveness if widely used, as mosquitoes might have no alternative areas to forage in, and so overcome the barrier tendencies.

Another approach is to try to treat for mosquitoes over a particular, defined area. Most of these space repellants are used to protect individuals inside the home or in the yard. They are most effective indoors. Outdoors, the insecticide particles disperse rapidly and, therefore, may not be effective. Many household aerosols contain synergized pyrethrum or a synthetic, pyrethroid equivalent such as allethrin, or resmethrin. This makes these products equivalent to pesticides used by SCVC, albeit without the application controls, and with the risks associated with pesticides assumed voluntarily (assuming there are no impacts outside of the immediate vicinity of the application).

Another common repellent choice is oil of citronella candles, torches, or coils. These can be used outdoors, but only in situations where there is minimal wind. One experiment found that ordinary candles had approximately the same repellent impact that citronella candles had (a reduction in bites of between one-quarter and one-half).

Enhanced predation

Predators touted for effectiveness against adult mosquitoes include dragonflies, birds (especially purple martins), and bats. These have been discussed above in Section 4, under "Biocontrols." Generally, except for dragonflies in a few select environments, enhanced predation except when mosquitoes are swarming appears to be ineffective as a means of controlling adult mosquitoes.

Traps

Special traps have been developed in the last few years that attract and catch large numbers of mosquitoes, thus potentially removing them from a radius around the trap. Brand names of such traps include the Mosquito Magnet, Mosquito Megacatch, the Flowtron Power Trap, the Dragonfly, the Lentek Mosquito Trap, The Lentek Eco Trap, Mosquito Deleto, and the SonicWeb. This technology is developing rapidly and there is considerable variability in the way these traps function. The vast majority of these traps use CO_2, produced either through the combustion of propane or via a CO_2 cylinder, and released at between 350 ml and 500 ml/min. The plume of CO_2 discharged mimics human exhalation and makes these traps specific for capturing blood-feeding insects. The CO_2 is often synergized with 1-Octen-3-ol, a derivative of gasses produced in the rumen of cows, to increase attractiveness by several orders of magnitude (any trap using octenol is subject to regulation by NYSDEC). The 1-Octen-3-ol is slow-released at a rate of approximately 0.5 mg/h. The traps also vary in the manner in which mosquitoes are trapped/killed. Some traps have a fan to suck insects into a collection chamber or bag, while others contain a glue board to catch the insects. Several of these traps claim to protect as much as an acre of land. Generally, however, mosquito control professionals are becoming convinced that trapping mosquitoes is not efficient enough to control mosquito populations sufficiently to prevent biting complaints. This is especially true when mosquito populations are dense and aggressive biters. The traps are gaining notice for their ability to capture good samples of the human-seeking mosquito population and so they may become standard surveillance equipment. For one, they capture day-flying mosquitoes when operated all day, and, unlike CDC and New Jersey traps, contain no lights to capture "trash" insects as well as the desired mosquitoes.

Arranging the traps so that they enclose spaces that warrant protection seems to be more effective than setting out an individual trap to protect the area surrounding the trap. The Long-

Term Plan tested the space enclosure concept experimentally. The results showed that CDC light traps within areas protected by mosquito traps actually caught more human-biting mosquitoes than did a CDC light trap located away from the array. This provides credibility to the scoffers who have said that the traps actually serve as attractants of mosquitoes, and do not reduce mosquitoes in the areas they are established. Still, anecdotal reports do continue to find that traps can be effective in some settings. Therefore, it may be that traps can work well in some areas with certain species of mosquitoes; such conditions did not occur when the County tested the devices, however.

7.3 Treatment Decisions

It must be emphasized that when adulticiding is being considered, it is in the context of IPM. Mosquito control has been undertaken through public education, source reduction (including aggressive, progressive water management programs), and larviciding. Adulticiding is being considered as the last means of achieving protection of human health and public welfare. It is certainly not the management tool of first choice for Suffolk County.

There are two possible conditions for adulticiding to occur under. One is when a health emergency is declared, and the other is for vector control purposes. In either case, a multivariate assessment of scientific surveillance information will drive the decision-making.

Typically, adulticide treatments are differentiated between those that are undertaken for the protection of human health and those that are needed for public health nuisance abatement to provide for relief of human discomfort. The planners of the County mosquito program have found it difficult to clearly separate these two kinds of event.

Nearly all human-biting mosquitoes in Suffolk County have some vector capability for the arboviruses that are the modern day health threats in the northeast US. Thus, control of these human-biting mosquitoes is undertaken to have some impact on the overall risk of disease. Actions taken to reduce the populations of human-biting mosquitoes in Suffolk County reduce the risk of disease transmission, and result in public health benefits beyond minimization of subclinical effects. In addition, there is a significant improvement in the quality of life for those

who live, work, or recreate where these mosquitoes live, when noxious mosquito numbers are reduced.

The Long-Term Plan therefore has a dual emphasis in terms of mosquito control. Public health protection emphasizes monitoring for pathogens among amplification vector populations, and controlling important bridge vector populations through source reduction (especially water management for salt marsh species), larval control where source reduction is not possible or was not effective, and, if a health risk assessment deems it necessary, adult control. There is significant overlap between this approach and the alleviation of severe public welfare effects. Suffolk County has significantly reduced pestiferous mosquito populations along the south shore through its current mosquito ditch maintenance program augmented by regular use of larvicides. The Long-Term Plan proposes to conduct more progressive water management in salt marshes, which should reduce the need for larvicide applications immensely, and be more consistently effective than the current program in eliminating those mosquito conditions that do not allow residents to remain outdoors during periods of the summer.

State and County Public Health Law (PHL) identify mosquito control and the reduction of mosquito habitat (such as standing water) as abatement of public health nuisance. A public health nuisance is a condition which adversely affects public health (irrespective of whether it causes fatal disease or some sublethal impacts). In this case it is the recognition of health effects from an ectoparasite (mosquitoes are grouped as such with pests such as lice, fleas, and bedbugs). Under State law, health officers have a duty to address the effects caused by these to the public. The presence of pathogens in mosquitoes is not required for this definition of public health nuisance, as the law implicitly recognizes there are health concerns that extend beyond the transmission of diseases such as WNV and EEE.

The Long-Term Plan uses the term "vector control" to describe adulticide applications in the absence of a detected pathogen. In general, "vector control" is interchangeable with "public nuisance control," as these instances of adult control take place under conditions where there is a low but imminent public health threat of the outbreak of serious disease (such as WNV or EEE), where the risk to the public cannot be said to be zero, and where sublethal impacts also occur.

The mosquitoes of Suffolk County develop in both fresh and salt water environments. In order for pathogens of present-day concern to become prevalent enough to pose a major health threat, they need to be amplified through avian reservoirs by fresh water mosquito species. The County, therefore, as it is allowed under regulations that protect important fresh water natural resources, conducts surveillance and control programs to reduce overall health risks. For EEE, it is clear that other mosquito species are needed to spread disease to people, and some of the most able of these species breed in salt water settings. For WNV, the cycling of the pathogen is less well understood, but quite a few fresh and salt water mosquitoes have been determined to be (or are suspected of being) human vectors. Therefore, the integrated control program that focuses on reducing these human-biting mosquito populations, in both fresh and salt water environments, clearly reduces overall risks of disease transmission.

The source reduction and larval control steps that reduce impacts to the quality of life for many, also serve to reduce the public health impacts that can result from mosquito-borne diseases. Waiting for the detection of pathogens in adult populations realistically limits disease control efforts to the least effective and potentially most environmentally harmful means of mosquito control, which is the use of adulticides. Reducing control measures in salt marsh environments because they provide major quality of life benefits will also increase overall disease risks, as the same species that bedevil south shore residents most also can transmit fatal disease. This, thus, is the basis for the assertion that program elements addressing quality of life and disease risks cannot be practically separated.

It is an axiom of many of the adulticide events that will occur under this Long-Term Plan that the alleviation of severe human discomfort has public benefits, including the reduction of health risks. Justifications for understanding the public benefits of such a stand include the personal benefits received by many in the target area, and also the perceived economic benefits that may accrue too many across the County. Tourism and related outdoor activities are important economic factors. All residents understand the value of the waterfront and related industries, that outdoor recreation such as golf and sightseeing are important summertime activities, and probably comprehend that the summer East End-Fire Island resort communities depend on those visiting and renting there being able to go outside when they wish.

This public policy is supported by the determination that there is little to no human health cost to the control of mosquitoes, using modern pesticides, at the rates currently planned for across the County. This is based on literature review, as well as through quantitative risk assessment (use of a model, etc.). The model of potential environmental impacts shows there can be some effects from the use of these chemicals, especially to some aquatic organisms and to night-flying insects. Nonetheless, use of them is to be pursued due to four mitigating factors. One is that the model quite probably overestimates environmental exposure to the agents, as locally collected information is making it clear that many of these compounds degrade more quickly than was ever anticipated. Secondly, the "bee" model for flying insect impacts, which relies on understanding impacts to bees, is probably not appropriate for considering impacts under these conditions. Bees do not fly at night, and these adulticides do not have residual effects. Bees are very sensitive to pesticides, more sensitive than most large flying insects; it is generally found that ULV mosquito applications tend not to reach toxic levels for most flying insects, as finer droplets do not deliver fatal doses. Secondly, the impact is limited in duration, as the community modeling suggested that aquatic species recover over the winter when no pesticides are applied, and some limited sampling in California found near immediate recovery for airborne insects. Thirdly, the amount of the County impacted by these chemicals is limited. The acreage of adulticiding for 2003 (a year when more pesticide than average was applied) was 34,650 acres compared to a total area of the County of 655,632 acres, meaning adulticiding in a high application year affected approximately five percent of the land area of the County. Another means of quantifying the limited exposure to the adulticides is that the "coastline" (counting streams and rivers) affected by adulticide use in 2003 was 220 miles of 1,852 miles County-wide, which constitutes approximately 12 percent of the coast (please note that these fractal kinds of measurements of coastlines vary considerably depending on scaling and other issues, and so should be understood to be very approximate measures). Many of these applications, because they occurred by truck or hand-held methods, had limited means for impacting aquatic environments due to intentional setbacks and also natural barriers that exist between the point of application (generally, a road) and the waterways.

Under a declared health emergency, the benefits associated with pesticide use include disruption of transmission of disease. The adulticide treatments are not made wherever indications of

disease are found, but rather where the risk factors indicate that the greatest possible risk is located.

Control decisions are not made merely on the number of mosquitoes, or the amount of human-biting that is occurring. These are important issues, but they are not definitive. Other information is required in order to determine if adult control is necessary:

- Species of mosquitoes present, from trap data
- Relative numbers of mosquitoes, by species, from trap data
- Population trends, from past data sets and control sites
- Aggressiveness of the mosquito population, inferred from trap data, based on species composition, based on complaint logs, and/or from landing rates
- Activity pattern of the species of concern (preferred feeding habits, resting habitats, etc.), from trap data
- Presence or absence of virus, from laboratory analysis of mosquitoes, dead birds (may no longer be realistic), sentinel birds, and/or wild avian surveillance, or the presence of human cases
- Analysis of the risk posed by the particular virus, based on professional judgment and CDC-NYSDOH guidance
- Parity of mosquitoes
- Bird migration patterns
- Current weather and short-term weather forecasts
- Long-term weather trends (time of year considerations)

Not every decision can have or needs to have a complete information set, and sometimes decisions may be tentatively made and then confirmed based on immediate data collection. The

kinds of applications that have historically been made will be revisited in light of the Long-Term Plan decision process, to illustrate how the process should function.

There are several areas in the County, mostly along the south shore, that typically experience inundations by broods of salt marsh mosquitoes several times in a year. It is intended that progressive water management will reduce the number of these incidents. Experience in other jurisdictions indicates that fish predation on larvae is more consistent than the effects of larvicides. Larvicides, if properly applied, should provide a great deal of control. However, sometimes the applications are adversely affected by weather, or tides may wash the pesticides off the marsh.

Knowledge of the mosquito broods comes to SCVC management in several ways:

- Reports from field crews prior to the outbreak, suggesting large numbers of larvae were present on the salt marsh (as a prelude to larviciding)
- Follow-up reports from field crews conducting larval surveillance on the marshes, indicating high numbers of biting adult mosquitoes on the marshes
- Increases in biting complaints from the community (these are logged and mapped by SCVC)
- Requests from elected officials (mayors, legislators and others) or community groups.
- New Jersey light trap data, indicating increases in *Oc. sollicitans* numbers in the sentinel traps

These events can sometimes be foretold, based on tide predictions of higher tides. However, the microtidal nature of the South Shore Estuary means that the highest water heights in the marsh (and therefore greatest marsh inundations), which precede the largest larval hatchings, often result from storms, and not necessarily lunar tides.

All complaints are followed up. Therefore, field crews will be dispatched to the areas where complaints are being logged, and will confirm (or not) that an infestation has occurred (people with party or holiday plans have been known to try to arrange for prophylactic applications to

ensure no mosquito disruptions). Informal landing rate tests across open fields are a good test for the presence of *Oc. sollicitans* during the day. If trap counts are excessive (at least 25 biting females per trap night, compared to a more usual zero to five count, in New Jersey light traps, and at least 100 biting females in a CDC light trap), and mosquitoes have been confirmed, the general area where the infestation is occurring is mapped, based on complaints received and the follow-up visits by field crews. Since truck applications are the typical means of responding, the road network of the area is used to determine the potential boundary of the application. Weather forecasts will be accessed to determine if conditions seem to be acceptable for a potential application, and to ensure a cold front or other storm situation will not occur to eliminate the need for the application. It is also assumed that the time of year indicates that the infestation is not about to become less due to cooler temperatures, as might be the case in September or later in the season, or in May or early June (mosquito activity slows with decreasing temperature, and rises with increasing temperatures). Population trends for the particular area will be observed to ensure that typically these conditions do persist (most of the areas where such control treatments are considered are well-known to SCVC administrative staff). No-spray addresses and key environmentally sensitive areas are factored in, and then the application area is noticed, so that an application can occur the next evening.

At this time, the QA/QC team should locate a suitable area in or near the center of the application block, and set up a baited CDC trap for confirmatory sampling. This trap would also be used for baseline data as a measure of treatment efficacy. Another trap, outside but near to and in a somewhat similar setting, could be established for a control site. In the morning, the two traps would be collected. The species and number of biting mosquitoes would be noted. A target for the decision to continue with application plans would be the presence of 100 or more biting mosquitoes in the CDC trap of interest. Anything less than this (assuming the trap functioned properly) should lead to application cancellation; a notable shift in the speciation of the trapped mosquitoes requires reassessment of the application decision.

Assuming that the trap confirms the decision, and the weather is appropriate, the application will occur on the second evening. The next night, CDC traps would again be set, and the collected data used to calculate the efficacy of the application. The intent of the control program is to reduce targeted species' numbers by an order of magnitude (measured trap counts, as adjusted by

the control results, would be expected to be 90 percent less than the original counts). These actions are intended to reduce impacts to the quality of life experienced in the neighborhood, and also to reduce disease risk by eliminating older mosquitoes from the available population. Breeding may also be slightly curtailed (but unless the marshes are also targeted, not enough of the salt marsh mosquito population will be killed to seriously impact overall breeding). Populations out on the marshes can only be successfully curtailed through effective water management and larvicide applications.

It is possible that areas outside of typical locations impacted by biting mosquito problems will appear to need treatment. In these cases, initiation of recognition of a problem will probably begin with complaint calls, and continue with follow-up on the calls. It is less likely a set New Jersey light trap will be set conveniently to assess the problem, and so the analysis may not proceed quite as quantitatively as described above. It is all the more important to analyze overall mosquito population trends for this season and previous seasons, in these cases, and to set the pre-application CDC light traps, and carefully analyze the data from those traps prior to confirming any application decision.

Figure 5 illustrates the decisions that are made to reach a vector control application decision. The term "professional judgment" is used to show that the decision most often involves weighting the factors that appear to indicate that control is necessary in light of those factors that indicate control is not necessary.

The steps that occur to determine whether a treatment occurs or not can be summarized under four general criteria:

1. Evidence of mosquitoes biting residents (there is no problem unless people are affected):
    - Service requests from public - mapped to determine extent of problem
    - Requests from community leaders, elected officials
2. Verification of problem by SCVC (service requests must be confirmed by objective evidence):

- New Jersey trap counts higher than generally found for area in question (at least 25 females (human-biting species) per night.
- CDC portable light trap counts of 100 or more human biting female mosquitoes.
- Landing rates of one per minute (generally measured over a five minute period).
- Confirmatory crew reports from problem area or adjacent breeding areas.

3. Control is technically and environmentally feasible (pesticides should only be used if there will be a benefit):

   - Weather conditions predicted to be suitable (no rain, winds to be less than 10 mph, temperature to be 65ºF or above).
   - Road network adequate and appropriate for truck ULV.
   - "No- treatment" wetlands, wetlands and open water buffers, and no-spray list members will not prevent adequate coverage.
   - There are no issues regarding listed or special concern species in the treatment area.
   - Meeting label restrictions for selected compounds (such as avoiding farmland) will not compromise expected treatment efficacy.

4. Likely persistence or worsening of problem without intervention (pesticides should not be used if the problem will resolve itself):

   - Considerations regarding the history of the area: is it a chronic problem area?
   - Will the problem spread beyond the currently affected area absent intervention, based on the life history and habits of the species involved?
   - Crew reports from adjacent breeding areas suggest adults will soon move into populated areas.

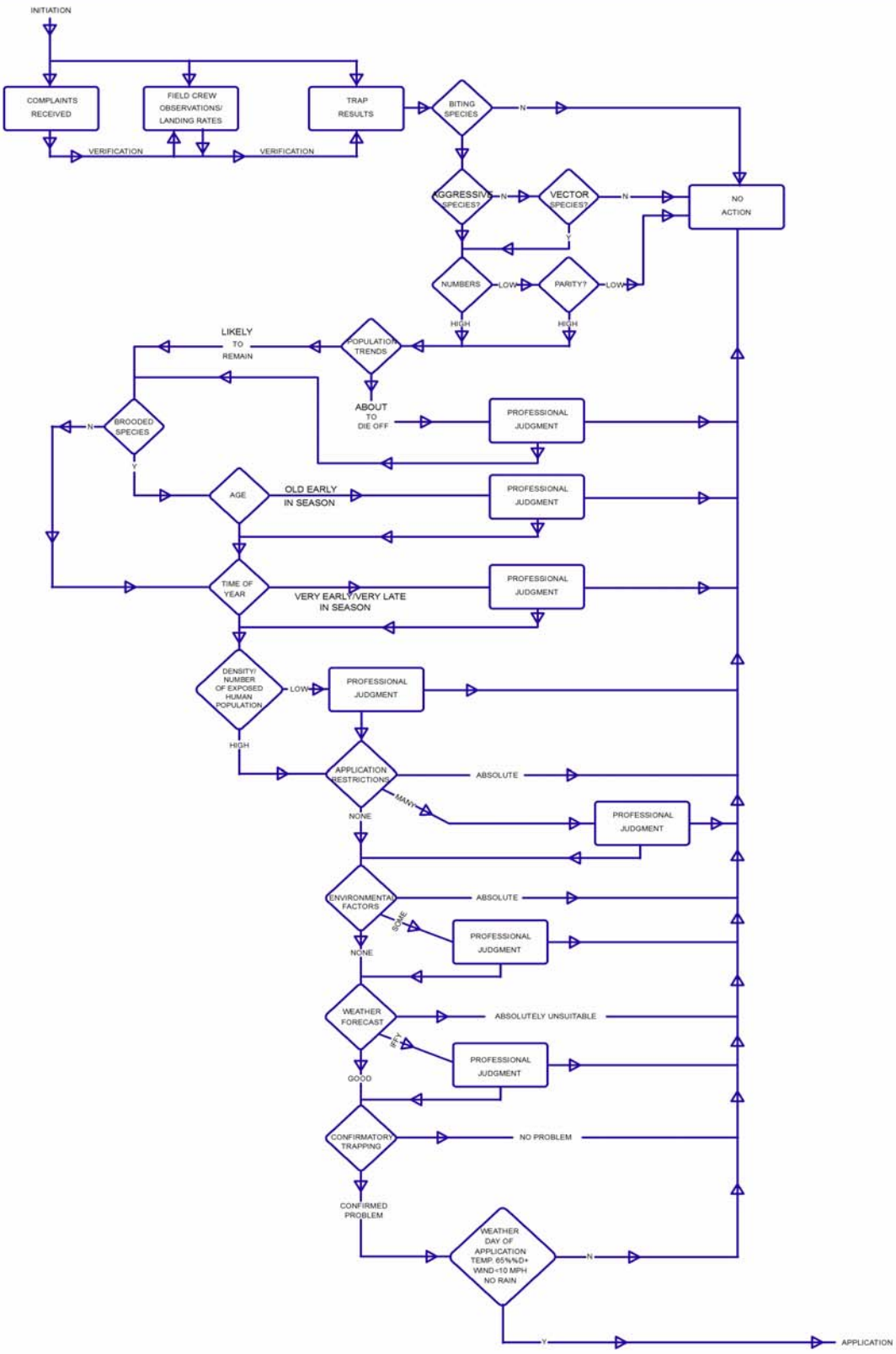
- Life history factors of mosquitoes present – if a brooded species, is the brood young, or is it naturally declining?

- Seasonal and weather factors: cool weather generally alleviates immediate problems, but warm weather/onset of peak viral seasons exacerbate concerns.

- If the decision is delayed, will conditions prevent later treatment? Or will adverse weather conditions remove most people from harm's way?

In essence, criteria 1 and 2 are necessary thresholds which must be met, prior to a treatment being considered. With enhanced surveillance, there will be rigorous, numeric validation of mosquito control infestation near a potentially affected population in all cases. Treatment will not occur unless criteria 1 and 2 are satisfied through a combination of surveillance indicators, although not all surveillance techniques may be feasible in every setting and situation.

Criteria 3 and 4 are "treatment negation" criteria. If certain conditions are met, treatment will not occur, even if it would otherwise be indicated by criteria 1 and 2. Careful records on criteria/thresholds (and related conditions) which trigger each treatment will be kept, for every adulticiding event.

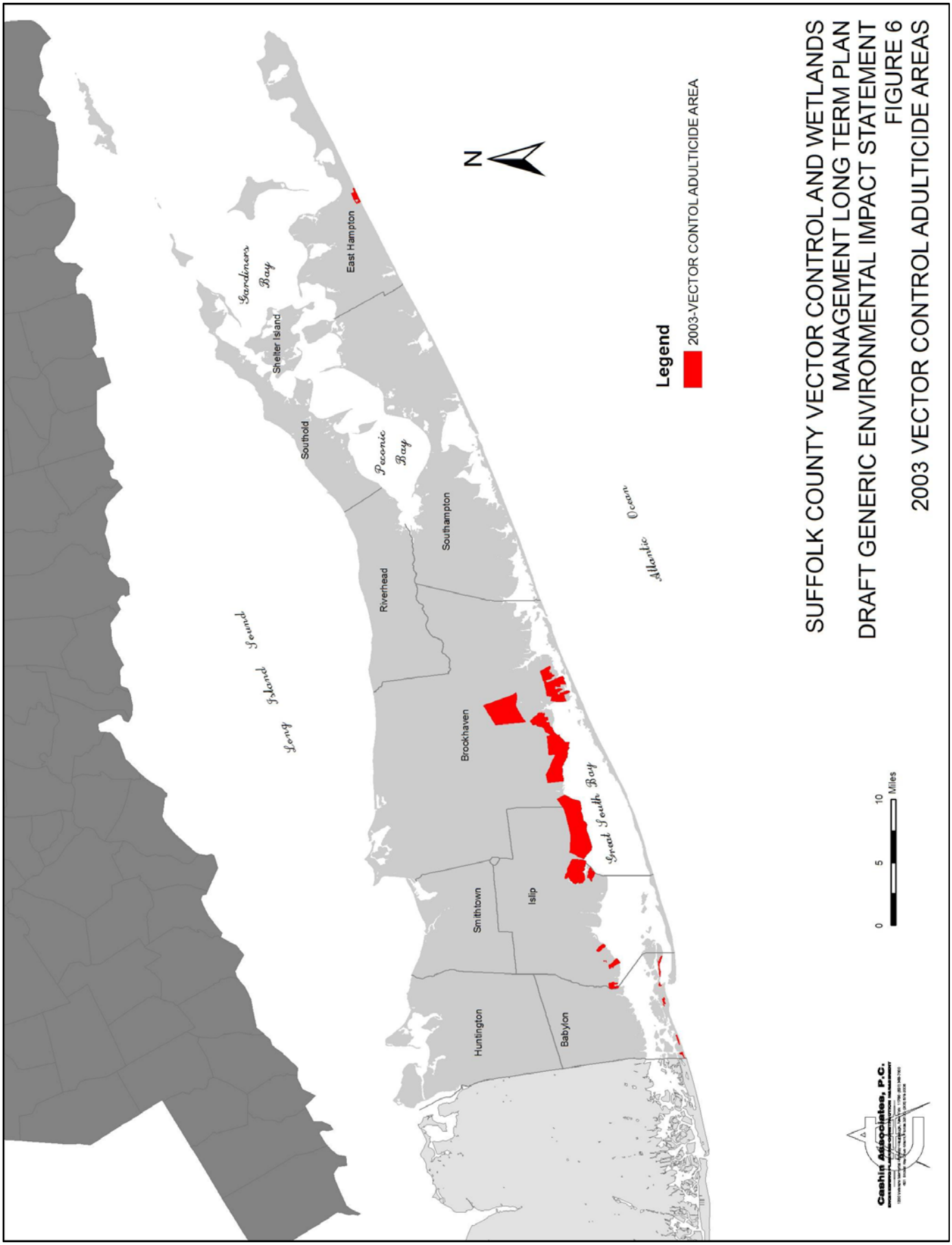


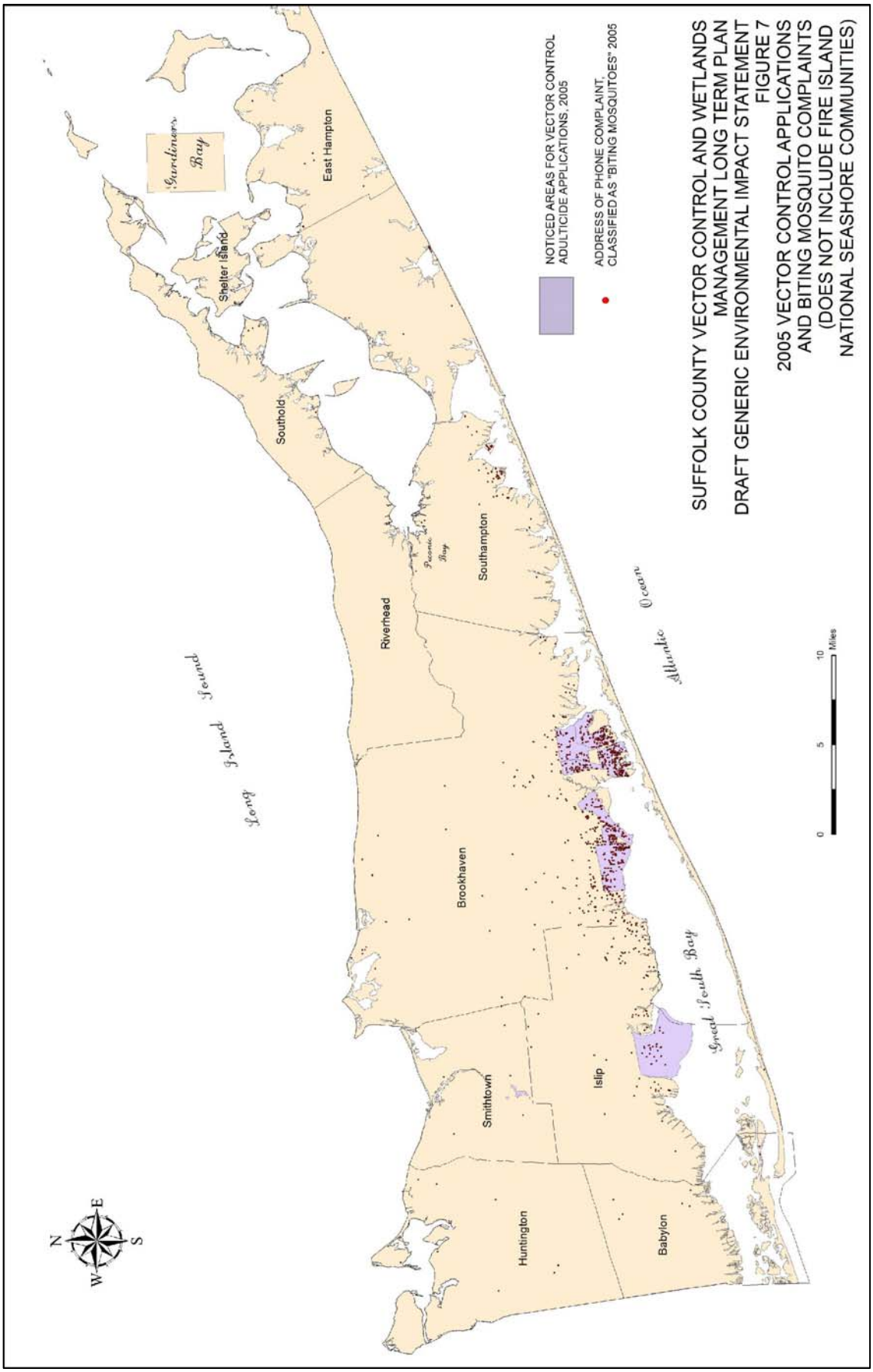
SUFFOLK COUNTY VECTOR CONTROL
AND WETLANDS MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 5
VECTOR CONTROL DECISION TREE

Table 20 lists the acreages for vector control treatments (outside of the FINS communities) for 2000 to 2004 (based on area identified through public noticing, not on actual applications, which were smaller – but are difficult to separate out). Figure 6 displays the areas noticed for treatment for Vector Control reasons in 2003 (the year with the most treatments post-2000). These data support the contention that Vector Control adulticide treatments constitute a relatively small part of SCVC's activities, and affect a relatively small portion of the County. Figure 7 shows the relationship between complaints received and areas noticed for treatment in 2005, which demonstrates that complaints do not guide Vector Control decision making currently, and will not do so under the Long-Term Plan.

Table 20. Acres Treated with Adulticide under Vector Control Determinations (FINS Communities not included)

Year	Vector Control Adulticide Acreage
2000	66,400
2001	27,600
2002	5,850
2003	34,650
2004	20,300


SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS
MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 6
2003 VECTOR CONTROL ADULTICIDE AREAS
Legend
2003-VECTOR CONTOL ADULTICIDE AREA
N
0
5
10
Miles
East Hampton
Gardiners Bay
Shelter Island
Southold
Peconic Bay
Southampton
Riverhead
Long Island
Atlantic Ocean
Brookhaven
Great South Bay
Smithtown
Islip
Huntington
Babylon
Cashin Associates, P.C.


SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS
MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 7
2005 VECTOR CONTROL APPLICATIONS
AND BITING MOSQUITO COMPLAINTS
(DOES NOT INCLUDE FIRE ISLAND
NATIONAL SEASHORE COMMUNITIES)
NOTICED AREAS FOR VECTOR CONTROL ADULTICIDE APPLICATIONS, 2005
ADDRESS OF PHONE COMPLAINT, CLASSIFIED AS "BITING MOSQUITOES" 2005
0
5
10
Miles
Long Island Sound
Atlantic Ocean
Great South Bay
Peconic Bay
Gardiners Bay
Shelter Island
East Hampton
Southold
Southampton
Riverhead
Brookhaven
Smithtown
Islip
Huntington
Babylon
N
E
S
W

Fire Island Communities

Historically, the County has conducted extensive vector control operations to preserve quality of life and provide a degree of health risk reduction. The exact dimensions of such programs under the Long-Term Plan are still being determined by the County and NPS. In broad outline, any program involving adulticide use in the communities on FINS will conform to the same general parameters as described immediately above:

- All decisions will be based on surveillance data. These data will be developed from traps, community reports and complaints, and site-specific testing of some form.
- Source reduction steps will be implemented, as possible.
- Larval control will also be used, as is permissible under NPS regulations.
- Community input into determinations of the suitability of vector control adulticiding in particular areas will be sought (adult control in the case of a declared health emergency is at the discretion of the Commissioner of the Department of Health Services in consultation with federal officials, and such decisions made to protect the public health are not subject to community concerns).

The County and FINS will increase the level of public education and outreach in the communities. These communiqués and meetings will stress the ability of individuals to control their exposure to mosquitoes, mosquito bites, and mosquito-borne diseases. Housekeeping measures that limit habitat, use of repellents, and other mosquito avoidance steps will be emphasized. It is possible that updated, more descriptive accounts of health risks may be generated for community members and visitors to the Seashore. This step may be required due to restrictions imposed on mosquito control activities in FINS, due to its special status and conditions imposed according to the mission of NPS.

The FINS-specific plan will be generated by the County, and is subject to appropriate reviews under the National Environmental Protection Act (NEPA), as any mosquito control activities in FINS will require a special-use permit from NPS.

Declared Health Emergencies

Control decisions under a declared health emergency are made differently. SCDHS is responsible for ensuring that the risk assessment has been properly conducted, and reviews the operational plan proposed by SCVC to meet the required risk reduction. The risk assessment first requires that mosquito-borne disease has been detected in the County. On rare occasions the problem has been malaria; however, the modern mosquito-borne diseases of concern are arboviruses. The most prominent of these, and the ones most likely to be detected in the County, are WNV and EEE.

The County's disease management protocol is based on the NYSDOH four-tiered WNV response strategy. It differs is some minor respects from that overall approach, but essentially follows the overall strategy. Table 21 summarizes the NYSDOH WNV response strategy.

Table 21. NYSDOH Four-Tiered WNV Strategy

Tier	Circumstances	Response
I	No historical or current evidence of virus No neighboring Health Unit with historical/current evidence of virus	Level 1 education campaign Enhanced passive human/bird surveillance Consider adult mosquito surveillance (species, distribution) Lower priority for lab testing Consider larval surveillance Consider local environmental assessments Consider local disease risk assessments
II	Historical evidence of virus Neighboring Health Units with historical evidence	Level 1 enhanced education program (general community & provider community) Local environmental assessments Local disease risk assessments Active human (if evidence in-unit)/bird surveillance Larval surveillance Larval habitat source reduction Larval control Adult surveillance and lab testing
III	Current virus isolation/evidence of infection in individual locations	Level 2/3 education program (general public & provider community) Active human/bird surveillance Larval surveillance Larval habitat source reduction Larval control Adult surveillance and lab testing Adult control, ground application
IV	Current virus isolation/evidence of infection in multiple locations	Level 2/3/4 education program (general public & provider community) Active human/bird surveillance Larval surveillance Larval habitat source reduction Larval control Adult surveillance and lab testing Adult control, ground application

Because WNV and EEE have been historically detected in Suffolk County, the County essentially begins each mosquito season in Tier II of the NYSDOH tiered approach.

Over the period 2000 to 2004, the signal of WNV presence in birds was finding dead crows that tested positive for virus. It appears that many of the susceptible crows have died from the disease, and in 2005 the survivors and their off-spring did not readily perish from WNV, at least as often and in as timely a fashion as they used to. Dead crow discoveries still correlate with positive pools of mosquitoes. However, in the 2000 to 2004 time period, the dead birds were almost always found before positive pools were detected (in a general geographic area). In 2005,

the finding of dead birds and determination of positive pools were mostly synchronous, and the sentinel value of dead birds was tremendously reduced.

This suggests that the development of new sentinels would be advantageous. Possibilities include sentinel chicken flocks (an approach that did not succeed when first tried in the County, but which has been successful elsewhere in the country), netting adult birds, or taking blood samples from nestlings. The latter holds great surveillance potential for early in the season, as the birds are sessile, and there is no potential of being seropositive from earlier exposure, or exposure elsewhere. A positive result would indicate that virus is circulating in that immediate area. However, nestlings often leave the nest just as WNV becomes of greatest concern (early August), which would mean changing surveillance tools at a key moment. In addition, permit issues make this kind of surveillance very difficult to administer. Nonetheless, the County should seek to develop some new means of conducting sentinel surveillance for WNV. Whatever method is selected, testing of these samples could continue to occur in-house, with some samples sent to NYSDOH in Albany for confirmation and more inclusive general viral scans.

If no alternative bird surveillance tool is developed, the County will need to step up its use of baited CDC traps, collecting more samples, more frequently, and from many more locations. The current proposed expansion of initial CDC trap setouts is relatively modest (from 27 traps to 35, and would not suffice to replace dead birds as an efficient means of detecting WNV County-wide). Currently, baited CDC traps are set at fixed stations in areas where EEE and WNV have reoccurred, and more are set to investigate bird deaths and positive bird samples. Gravid traps are also set to particularly target *Cx. pipiens* (for WNV surveillance). Absent bird deaths to target sampling, means of generally conducting surveillance across the entire County will need to be established. This will require some method of increasing the density in both time and space of the CDC trap network. Increasing the number of CDC trap samples collected is very labor intensive, both in terms of managing the traps (set-outs and sample collections) and in processing the collected samples. The nature of mosquito-borne disease is also that a low infection rate in mosquitoes can result in very high infection rates in target species, since a few positive mosquitoes can infect multiple targets, so that sampling mosquito pools is often less efficient at

identifying areas where infectious agents are present and circulating. For these reasons, identification of alternate bird sampling methodologies is preferable.

If surveillance reveals the presence of WNV (birds or mosquito pools), the County will petition to the State Commissioner of Health for a declaration of a Health Threat (sometimes also called a Health Emergency, but here distinguished from the local declaration by the Suffolk County Commissioner of SCDHS). This allows the County to apply for reimbursement of certain expenses in SCDHS relating to mosquito control, and places SCVC formally under the direction of the Commissioner of SCDHS. It is also a necessary first step prior to any declaration of a Health Emergency. This also moves the County to Tier III of the NYSDOH tiered response strategy.

A health threat declaration will also be sought in sampling results from *Cs. melanura* pools shows that EEE is amplifying in bird populations. This is signaled by detection of a *Cs. melanura* positive pool from samples sent to Albany for analysis.

The declaration of a health threat will also be accompanied by public education, through SCDHS press releases and web site publications. These are intended to draw attention to the heightened state of concern regarding mosquito-borne disease. Localities where virus has been detected will be called out specifically, but the outreach is intended to remind all Suffolk County citizens and visitors of the steps that can be taken to minimize the chance of being bitten by mosquitoes. In addition, SCDHS will contact its physician and hospital reporting network, and touch base with its local veterinarians. This ensures that any human or sentinel animal cases of mosquito-borne disease are promptly reported.

Detections of clusters of positive WNV pools for *Cx. pipiens* would signal the potential for adulticide control. In that case, the presence or absence of potential bridge vectors would be an important consideration, especially if the bridge vectors tended to have a higher parity rate. For flood water mosquitoes, a determination as to whether a brood was waning naturally, and need no control for numbers to be of little concern, would also be a factor, although not necessarily a compelling one. With bridge vectors, older mosquitoes are much more dangerous than young mosquitoes, so a large population of nulliparous mosquitoes is much less risky than a small population entirely populated by blooded mosquitoes. Time of year is important, as it has been

suggested that *Cx. pipiens* changes its feeding habits after the first week of August or so, and feeds more regularly on humans. This makes it a more dangerous mosquito, especially as the species (in general) transitions from bird feeding to human feeding (increasing the potential to pass virus along). In late summer, as night temperatures drop, *Oc. sollicitans* begins feeding more commonly during the day. This makes control harder, as the mosquito is less likely to be flying when the insecticide would be applied. Thus, late summer-early fall adulticiding is less common for *Oc. sollicitans* control purposes. These conditions move the County to Tier IV of the NYSDOH tiered strategy.

Another factor considered in control decisions is the size of population (and its composition, if greatly different from the County as a whole) in the near vicinity of the problem. Generally, the more people potentially exposed to the disease threat, the greater the likelihood of an adulticide application – although if the perceived risk is exceptionally high, then the number of people exposed is not as much a factor (i.e., 100 percent infection rates for five people means five cases of disease, and a 0.1 percent infection for 5,000 people also results in five cases of a disease). The community of Ridge, for example, includes several very large retirement villages. A disease such as WNV that seems to make older people sicker than it makes younger people is of more concern here than in other areas of the County.

If positive results occur in a bridge vector pool, then this too signals a potential need for adult control. If the virus were to be detected in *Oc. sollicitans*, especially, given its very aggressive biting habits and generally large numbers, concerns would be raised. The age of the brood, the time of year (control is more difficult late in the year when the mosquitoes fly at night as less often), and weather patterns (will the mosquitoes be killed by colder weather, or is the heat likely to make them even more active) all need to be factored into the decision.

For EEE, the threat of a bridge vector brood near a cycling center is a strong impetus towards declaration of a health emergency. Generally, Suffolk County has focused on EEE control in the near vicinity of the amplification area. Information gathered through the Long-Term Plan project provides support for the benefits of controlling *Oc. sollicitans* in all areas when EEE threatens, especially where coastal red maple or Atlantic white cedar swamps occur. *Oc. sollicitans* has been persuasively portrayed as the most dangerous and most effective potential

vector for EEE. The need to control *Oc. sollicitans* and other bridge vectors generally was underscored through discussions of the potential for dispersing young birds to carry the virus to anywhere along their migration route from natal swamps (where they may have contracted EEE). Any dead horses, or dead farmed pheasants or emus, would also signal the need for a health emergency declaration to address EEE, as all of these quickly succumb to the disease. Disease in horses is of special concern, as it signals presence of the virus in a bridge vector.

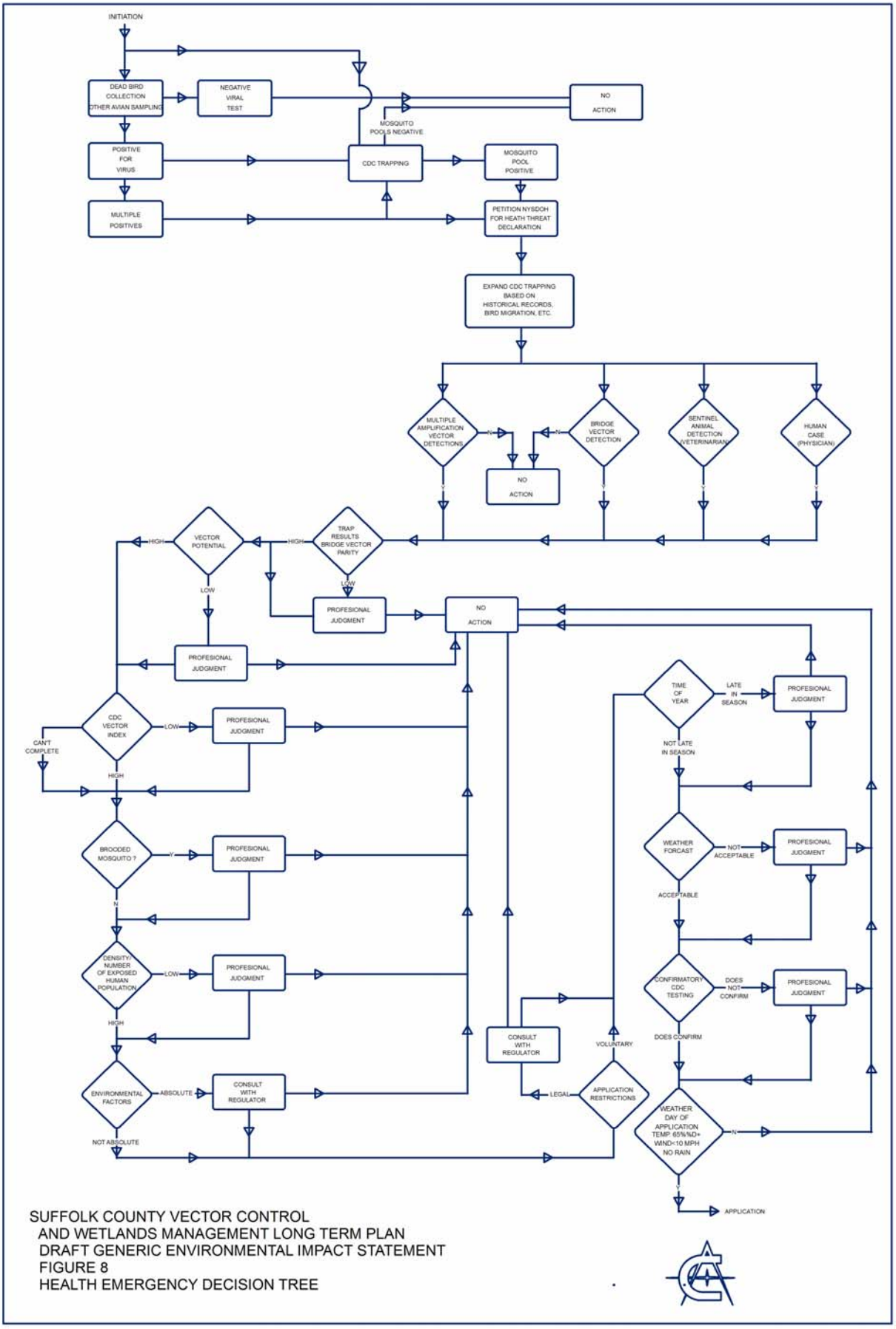
Working with SCVC, SCDHS would determine the best application zone, and determine the most appropriate application approach, based on the target mosquito. Hitherto, Suffolk County has focused its control efforts on bridge vectors, meaning that applications are conducted primarily right after sunset, when nearly all important mosquito species are active. Where *Cx. pipiens* is clearly the mosquito of concern, the timing of an application may be retarded to effectuate a better control on this later-flying mosquito. The determination of the most appropriate treatment time will be made by the Commissioner of SCDHS, in collaboration with technical experts from SCDHS and SCVC. The target area will be based on surveillance data, tempered by natural features (although a waiver from fresh water setbacks will be received for any disease threat application, major bodies of water serve as natural barriers to mosquito migration and so there is no need to apply pesticides over them needlessly) and label restriction areas such as croplands, if they can be avoided. Notices will be filed, and the expedited NYSDEC emergency authorization process pursued. Generally, staff from NYSDEC will make themselves available on very short order to enable a coordinated consultation regarding the proposed application zone to address sensitive species and habitat concerns.

Similarly to vector control applications, the QA/QC team will set out a minimum of two sets of baited CDC traps. Not only will these traps serve as efficacy measures for the treatment to follow, but sampling the trapped populations for species and parity can reinforce – or cause re-evaluation – of the application decision. Parous mosquitoes of concern should be present to cause the application to move forward – although it should be understood that at any given time approximately 50 percent of a *Cx. pipiens* population is parous. Pools from the traps will also be tested for virus presence, although if State facilities are used the results will not be received in a decision-timely manner. Efficacy will be at least partially determined if parity is lower after the

application, and, if pathogens were detected in pools before the application, a measure of application efficiency will be if they are or are not detected in pools after the application.

It must be understood that all decisions to apply adulticides in Suffolk County are made in the context of an IPM system. Adulticide applications are always the last, least desired control measure. Great efforts will have been made to avoid their use, beginning with public education, source reduction (including water management), and larval control steps. The decisions are not made arbitrarily, but in light of collected data from a surveillance system that has been bolstered from one described as among the best in the country. Adulticiding will only be undertaken to avoid worse consequences, in full knowledge of the benefits and risks associated with the action. These considerations mean that the County decisions clearly comply with the letter and the spirit of all Federal and State guidelines issued to help managers make the best possible choices under difficult conditions.

Figure 8 illustrates the decision-making process followed when adult control is being considered as a Health Emergency measure.



SUFFOLK COUNTY VECTOR CONTROL
AND WETLANDS MANAGEMENT LONG TERM PLAN
DRAFT GENERIC ENVIRONMENTAL IMPACT STATEMENT
FIGURE 8
HEALTH EMERGENCY DECISION TREE

7.4 Selected Pesticides

Pesticides selected by Suffolk County for adulticide control under the Long-Term Plan are all suited for ULV treatments. The quantitative risk assessment and modeling (based on EPA guidance documents) indicates no to little detectable human health impacts, and all have relatively insignificant ecological impacts. The ecological impacts are further mitigated by the relatively small areas that pesticides will be applied in, and the distinct probability that the model (which is based primarily on laboratory testing) overestimates the concentrations of pesticides actually delivered to aqueous environments by several factors, based upon testing conducted in association with this project. That being the case, it is clear that a model recalibrated with empirical data would confirm the somewhat tentative findings of the Caged Fish study, and find little to no impacts to the ecosystem.

In addition to ULV applications, malathion is approved in New York State by NYSDEC for thermal fogging. Malathion, permethrin, and sumithrin are also approved by NYSDEC for hand-held applications.

Resmethrin, sumithrin, and permethrin, which are synthetic pyrethroids, and malathion, an organophosphate, have all been selected as the primary adulticide agents for the program. The pyrethroids will all be used in formulations that use piperonyl butoxide (PBO) as a synergist. Natural pyrethrum has received a limited selection as a secondary pesticide, to be used for resistance purposes, and because it has label clearances for use over cropland. Any selected pyrethrum formulation will also contain PBO as a synergist.

Resmethrin is to be the primary material for truck and aerial ULV applications. This is based on its record of effectiveness, and the results of the risk assessment (which showed that impacts to human health or the environment were unlikely). Its rapid degradation in the environment provides a margin of safety in avoiding adverse impacts.

Sumithrin is to be the primary material for hand-held applications, as the label for this product (Anvil) allows for use with small aerosol droplets, while resmethrin (Scourge) does not, currently. Because of the similar risk profile found for sumithrin compared to resmethrin, sumithrin would be an acceptable alternate if resmethrin were not available.

Permethrin had higher ecological risks associated with its use, and also has label setback requirements that make it less practicable for use in shoreline settings. However, permethrin is a widely produced product, and so is likely to remain available if the other three pyrethroids were not due to market contractions.

Natural pyrethrum did not receive as extensive a review as the other pyrethroids in the quantitative risk assessment. It appears to have a similar risk profile. It degrades very rapidly, giving it a margin of error with regard to potential risks. Its labels also allow for application over crops, which is not the case for other pyrethroids. It is expensive (as compared to other pyrethroid products), and is sometimes not readily available.

Malathion is of a different chemical class than the pyrethroids (as an organophosphate), which means if pyrethroid resistance became an issue, it would be useful to have as an approved product. It also is labeled for thermal fogging, which is a useful application technique in some settings (underground structures or tire piles). It is technically more difficult to use as a ULV product, and the risk assessment indicated it has higher risks with regard to potential human health or ecological impacts than the other products. Malathion is identified in the Long-Term Plan only as a specialty tool, for instances where the other pesticides would not be effective or cannot be used.

Pyrethroids

The pyrethroids are synthetic pyrethrin-like materials widely used for insect control. Pyrethrins are natural pesticides harvested from some chrysanthemum plants (mainly *Chrysanthemum cinerarnaefolium*). Chemically, pyrethroids are esters of specific acids (e.g., chrysanthemic acid, halo-substituted chrysanthemic acid, 2-(4-chlorophenyl)-3-methylbutyric acid) and alcohols (e.g., allethrolone, 3-phenoxybenzyl alcohol).

Pyrethrins and pyrethroids have a similar mode of action — they work on the nerve axons by keeping open sodium channels used to propagate signals along a nerve cell. Initially, they cause nerve cells to discharge repetitively; later, they cause paralysis. These pesticides affect both the peripheral and the central nervous systems. When applied alone, pyrethroids may be swiftly detoxified by enzymes in the insect. Thus, some pests will recover unless the effect is

augmented. To delay the enzyme action so a lethal dose is accomplished for pest control, a synergist (e.g., piperonyl butoxide) is generally added to pyrethroid formulations to improve efficacy.

Pyrethroids are generally favored above malathion as adulticides. This is because the degradation of pyrethroids in the environment is so swift as to make it extremely difficult to cause any human or environmental impacts, and yet the pesticides still retain efficacy in killing targeted mosquitoes.

Resmethrin

Resmethrin is the preferred pyrethroid, and is generally the adulticide of choice for the Long-Term Plan because of its effectiveness and chemical properties. Resmethrin is a broad spectrum pyrethroid insecticide used for control of flying and crawling insects in homes, greenhouses, indoor landscapes, mushroom houses, and industrial sites, insects that infest stored products, and for mosquito control. It is also used for fabric protection, pet sprays, and shampoos, and it is applied to horses and in horse stables.

The risk assessment concluded, at the concentrations resmethrin might be applied in Suffolk County, no significant increases in risks for health or ecological effects would follow from its use. Resmethrin was identified as potentially impacting night-flying insects, although this appears to result from use of honey bees as the sentinel flying insect based on information availability. Honey bees appear to be more susceptible to impacts from pesticides than other large insects, and so their use may overstate risks. The effect is likely to be short-lived: sampling in California found that following some reduction in insect populations after adulticide events, the populations rebounded in a matter of days. In addition, to further mitigate the potential for any impacts, the Caged Fish study reported much lower concentrations of resmethrin in the water column than were used by the risk assessment model. The lower concentrations are apparently due to quick environmental degradation of the compound, which was not completely factored into the risk assessment method. In addition, the generally small area of the County that might be affected by resmethrin use should be considered. In 2003, when pesticide applications exceeded recent mean amounts, approximately five percent of the land area of the County was treated, accounting for approximately 12 percent of the County's shoreline. In addition, it is

anticipated that the gradual implementation of more progressive water management techniques could lead to a reduction in the need to apply pesticides for mosquito control purposes.

Sumithrin

Sumithrin (sumethrin, phenothrin) is currently used in hand-held adulticide applications (current NYSDEC interpretations of the resmethrin label do not allow resmethrin to be used in hand-held applications). This use would continue under the Long-Term Plan.

Sumithrin is a broad spectrum pyrethroid insecticide registered for use against mosquitoes in swamps, marshes, and recreational areas. Sumithrin can also be used to eradicate pests in transport vehicles such as aircraft, ships, railroad cars, and truck trailers, and for institutional non-food use, use in homes, gardens, and greenhouses, and on pets

The risk assessment concluded, at the concentrations sumithrin is applied in Suffolk County, no significant increase in risks for health or ecological effects would follow from its use. As with all of the pesticides considered by the risk assessors, the risk assessment found there might be impacts to night-flying insects. As discussed above, this appears to result from use of honey bees as the sentinel flying insect based on information availability. To further mitigate the potential for any impacts, the generally small area of the County that might be affected by sumithrin use should be considered.

Permethrin

One potential problem with resmethrin and sumithrin is that they are relatively low volume production pesticides. This means if the manufacturer discontinues the product for any reason, the program may be without alternatives that have been reviewed and determined to meet its needs. Therefore, two alternative pyrethroid/pyrethrin products have been identified as meeting the needs of the County, including permethrin.

Permethrin is a broad spectrum pyrethroid insecticide which is used against a variety of insect pests. It is used in greenhouses, home gardens, and for termite control. It also controls animal ectoparasites, biting flies, and cockroaches. Permethrin is additionally used to control insects on a variety of food and non-food products, including on nut, fruit, vegetable, cotton, ornamental, mushroom, potato, and cereal crops, and is the active ingredient in several topical anti-parasitic

formulations used in human and veterinary medicine. Lice control is a common use, for instance.

There are four isomeric forms, two cis- and two trans-, of technical permethrin. Product formulations can vary greatly in isomeric content.

The risk assessment concluded, at the concentrations permethrin might be applied in Suffolk County, no significant increases in risks for health or ecological effects would follow from its use. The bee model, which is the basis for this finding, may overstate risks, and sampling data from California indicates any impact is likely to be not measurable within days of the application. In addition, permethrin was found to have some potential to impact aquatic invertebrates. Sophisticated ecological modeling found that the loss of certain invertebrates would not have any greater ecological impacts (i.e., the effects did not propagate up the food chain). Additionally, longitudinal modeling suggested rapid recovery for any affected species, so that full ecological recovery would be expected by spring following any application the previous year. These results are somewhat expected, given that permethrin is not persistent in the aquatic environment and does not bioaccumulate to any significant degree. To further mitigate the potential for any impacts, the generally small area of the County that might be affected by permethrin use should be considered.

Pyrethrum

To add to the selection of pesticides available for County use, and to ensure the County has a product that is registered for use in agricultural areas should treatment there be required, pyrethrum has been added to the list of approved products. It is somewhat costly, however, and can be difficult to acquire during high demand periods.

Pyrethrum is a natural, botanical pesticide that is an extract of flowers from certain chrysanthemum species. The flowers are either dried or powdered, or their oils are extracted with solvents. The resulting pyrethrum extract or powder is composed of individual pyrethrins; including pyrethrin I and pyrethrin II, cinerins and jasmolins, which are the components that have insecticidal properties. Most of the pyrethrin pesticide products that are available also contain a synergist, such as PBO.

Pyrethrum was not as closely investigated as the other three pyrethroids. However, indications are that it is somewhat less toxic than the synthetic pyrethroids. This suggests that, at the concentrations it would be applied in Suffolk County, no significant increases in risks for health or ecological effects would follow from its use.

PBO

PBO is a derivative of piperic acid and, as discussed, is generally utilized as a chemical synergist in pyrethroid formulations. Pyrethroid products containing PBO are used to control mosquitoes in outdoor residential and recreational areas, as well as indoors to control insects such as fleas, ticks, and ants. Formulations of pyrethrins containing PBO are also used as a pediculicide to control body, head, and crab lice. PBO, in and of itself, at the concentrations modeled to result in the County from applications of PBO-containing pesticide formulations, was found by the risk assessment not to cause significant increases in risks for human health or environmental impacts. The pyrethroid/pyrethrin results of the risk assessment reported above included additive effects that may result because of PBO use as a synergist.

Malathion

Organophosphate pesticides consist of a broad class of chemicals used primarily in insect and pest control. These pesticides cover a wide variety of use categories, such as forests and woodlands, greenhouse food and non-food crops, livestock, seed treatments, oilseed and fiber crops, stored food and feed, terrestrial feed and food crops, structural uses, outdoor ornamental and indoor plants, plantscapes, and turf.

Malathion is a nonsystemic broad-spectrum organophosphate chemical that is used in agriculture and horticulture applications. Malathion has been widely used since the 1950s on raw agricultural products including edible grains, fruits, nuts, forage crops, cotton, and tobacco. Malathion has also been used to control parasites of livestock and domestic animals, through aerial applications in and around livestock barns, dairies, poultry houses, and food processing plants. Malathion has widespread use as a ground and aerial application to control Mediterranean fruit fly (Medfly) and mosquito populations. Malathion is used as a pediculicide in shampoos to treat head lice on children and adults.

Malathion contains approximately five percent impurities consisting largely of reaction byproducts and degradation products. As many as 14 impurities have been identified in technical-grade malathion, including isomalathion and malaxon.

Malathion possesses a relatively low acute toxicity compared to other organophosphates. The risk assessment concluded, at the concentrations that malathion might be applied in Suffolk County, that no significant increases in risks for health or ecological effects would follow from its use. Malathion was found to have the potential to impact aquatic invertebrates – a slightly greater potential than was found for permethrin. Sophisticated ecological modeling, of permethrin suggested that the malathion impacts, which were similar in scope, including short-term impacts to certain invertebrates, should not have any greater ecological impacts (i.e., the effects did not propagate up the food chain). Additionally, longitudinal modeling suggested rapid recovery for any affected species, so that full ecological recovery would be expected by spring following any application the previous year. These results are somewhat expected, given that malathion is not persistent in the aquatic environment and does not bioaccumulate to any significant degree.

It should be understood that public perception of the toxicity of malathion is based largely on work conducted on agricultural pest control applications. The label rates for malathion for use as a mosquito control pesticide are lower than for its use against general agricultural pests. Mosquitoes are more sensitive to pesticides than most other insects. This means that malathion is applied for mosquito control at much lower concentrations than it is for agricultural pest control, and so any potential impacts are much less as well.

Table 22 and Table 23 summarize the quantitative risk assessment findings for the selected adulticides (natural pyrethrum was only qualitatively reviewed, and so is not included in the tables).

Table 22. Summary of the Human Health Risk Assessment for Adulticides

Agents Considered	Most Critical Endpoint Considered	Pathway Considered Potential Risk	Locations with Potential Risk	Conclusion in Risk Assessment
Adulticides				
Resmethrin	incr. liver wgt, blood chemistry changes, behavioral effects	No pathways or populations presented acute or chronic risks of concern*	No locations had risks* of concern	The use of resmethrin products for vector control does not pose a health risk* under study conditions
Sumithrin	increased liver wgt and adrenal cortex toxicity	No pathways or populations presented acute or chronic risks of concern*	No locations had risks* of concern	The use of sumithrin products for vector control does not pose a health risk* under study conditions
Permethrin	neurological impairment	No pathways or populations presented acute or chronic risks of concern*	No locations had risks* of concern	The use of permethrin products for vector control does not pose a health risk* under study conditions
Malathion	cholinesterase inhibition, maternal toxicity	no acute risks*, some risks to RME child resident and adult community gardener	Davis Park only	Malathion does not pose a significant health risk to study area receptors
Degradates				
Malaxon	NA	NA	NA	
Isomalathion	NA	NA	NA	
Synergist				
PBO	reproductive and developmental toxicity liver and body wgt dec., laryngeal hyperplasia	No pathways or populations presented acute or chronic risks of concern*	No locations had risks* of concern	The use of PBO-containing products for vector control does not pose a health risk* under study conditions

* That is, predicted exposures were below levels of concern established by USEPA and/or others and so do not indicate that there is an increased risk of unacceptable impacts to human health from use of the pesticides under the conditions evaluated in this assessment

Table 23. Summary of the Ecological Risk Assessment for Adulticides

Agents Considered	Terrestrial Birds, Mammals, Reptiles	Terrestrial Insects	Aquatic Life	Comments	Conclusion in Risk Assessment	Role in Management Plan
Adulticides						
Resmethrin	No risk*	Risks to non-target insects, such as butterflies, bees, dragonflies; all locations	No risk*	Terrestrial insect risks used honeybees as surrogate. Endpt was maintenance of abundance.	Terrestrial insect risks can be mitigated by timing applications approp.	Primary material for truck and aerial ULV, based on effectiveness and results of risk assessment.
Sumithrin	No risk*	Risks to non-target insects, such as butterflies, bees, dragonflies; all locations	No risk*	Terrestrial insect risks used honeybees as surrogate. Endpt was maintenance of abundance	Terrestrial insect risks can be mitigated by timing applications approp.	Primary material for hand held ULV. Would be first choice if resmethrin cannot be used.
Permethrin	No risk*	Risks to non-target insects, such as butterflies, bees, dragonflies; all locations	Only chronic risk to individual aquatic insects/larvae and crustaceans in shallow water (e.g., daphnid, opossum shrimp, Eastern oyster)	Terrestrial insect risks used honeybees as surrogate. Endpt was maintenance of abundance	Terrestrial insect risks can be mitigated by timing applications approp. Aquatic risks will not result in community level impacts	Primarily will be used as an alternative for the other pyrethroids, due to setbacks and higher risks estimated in risk assessment.
Malathion	No risk*	Risks to non-target insects, such as butterflies, bees, dragonflies; all locations	Only risk to individual aquatic insects and crustaceans in shallow water bodies (e.g., stonefly, amphipod, mysid shrimp)	Terrestrial insect risks used honeybees as surrogate. Endpt was maintenance of abundance	Terrestrial insect risks can be mitigated by timing applications approp. Aquatic, community level impacts not expected	Since a different class than the pyrethroids, could be used if pyrethroid resistance becomes an issue. Label restrictions make it less useful for ULV and risk assessment indicates higher risk.

Agents Considered	Terrestrial Birds, Mammals, Reptiles	Terrestrial Insects	Aquatic Life	Comments	Conclusion in Risk Assessment	Role in Management Plan
Degradates						
Malaxon	NA	NA	NA	Not quantitatively evaluated due to lack of exposure, fate and toxicity data	NA	
Isomalathion	NA	NA	NA	Not quantitatively evaluated due to lack of exposure, fate and toxicity data	NA	
Synergist						
PBO	No risk*	Risks to non-target insects, such as butterflies, bees, dragonflies; all locations	No risk*	Based on evaluation of PBO containing products	Terrestrial insect risks can be mitigated by timing applications approp.	Combined with pyrethroids to maximize ULV effectiveness

* That is, predicted exposures were below levels of concern established by USEPA and/or others and so do not indicate that there is an increased risk of unacceptable ecological impacts from use of the pesticides under the conditions evaluated in this assessment

7.5 Formulations

Scourge 18-54 will be the resmethrin product used by the County. Product labels contain the signal word "CAUTION" (see Appendix 1 for information on signal words). The product will be applied either by ground or aerial ULV at label rates.

Anvil 10+10 will be the sumithrin product used by the County. It has a label that contains the signal word "CAUTION." Sumithrin will be applied primarily through hand ULV applications, although it may also be used for ground or aerial ULV uses.

Commercially available permethrin products include Permanone and Aqua Reslin. The patent has expired, and so brands are proliferating. The County has not yet selected a preferred provider. Permethrin labels may contain either the signal word "WARNING" or "CAUTION,"

depending on the formulation. Permethrin is not a primary use adulticide for the County, but rather will be used if other pyrethroids become unavailable.

Commercially available pyrethrum products include Pyrocide, and Pyrenone. The County has not yet selected a preferred product. Product labels contain the signal word "CAUTION." Formulations generally contain five percent pyrethrins with PBO at a one to five ratio. They are applied as a ULV application, and are expensive compared to other products, and sometimes are difficult to obtain because demand outstrips supply. Pyrethrum will be used for resistance purposes, and over agricultural areas, if required.

Fyfanon will be the malathion product used by the County. It is one of the most widely used adulticides in the country, primarily because of its lower cost compared with other approved adulticides. The label contains a "CAUTION" warning indicating that it is only a slightly toxic material. Malathion is generally used against all mosquito species of concern, primarily as a ground ULV application, needing no mixing or dilution. For thermal fog applications, malathion is diluted six to eight ounces per gallon with a suitable oil carrier, and applied at up to 40 gal./hr. with a vehicle speed of five mi./hr. (or at commensurately faster rates if the vehicle speed is greater). Malathion can be applied using ULV aerial application techniques, following label instructions. Malathion will primarily be used for resistance purposes, or if thermal fogging is necessary.

7.6 Application Methods

The County uses three application methods, with variations associated with several of the different means. In all instances, to address resistance concerns, and to achieve the best possible results, the County will apply the pesticides at the maximum rate allowed by the product label.

There are some general constraints on all application events. Low temperatures inhibit mosquito activity; SCVC has set 65 degrees F as the minimum for operations. Winds cannot exceed 10 mph, as mosquito activity is lower when conditions are windy, and the pesticides will disperse too quickly. Mosquitoes are not as active in the rain, and rain will remove pesticides from the atmosphere, making the application pointless. Therefore, rain is counterindicative for applications.

On Fire Island, where vehicle access is difficult, a golf cart type platform will be used to hand haul a London Aire Colt Hand Portable ULV Aerosol Generator to apply adulticides. This is a ULV treatment. Hand applications are only conducted for vector control treatments. Health emergency applications over Fire Island would most probably be conducted by helicopter, as the scope of the event would almost certainly exceed one community (the practical limit for hand-held applications). Please note that applying adulticides by aircraft is one way that the County's virus response plan differs from NYSDOH guidelines. State guidelines suggest using trucks to apply pesticides; Suffolk County prefers aerial applications in response to Health Emergencies (see below for a discussion of some of the factors that bear on this decision).

The planned hand-held application will be discussed by managers and applicators prior to the applicators leaving SCVC offices. The application route will be specified, along with any setbacks, no-spray properties, and other areas that will not be treated. The specific path to be followed will not be mapped, but will depend on operator judgment (resort communities present special problems such as parties and other congregations of people that need to be adjusted for in the field). Prior to initiating treatment, the crew would conduct spot larviciding as needed, and also conduct a landing rate survey to ensure *Oc. sollicitans* mosquitoes are still present.

The protocol to ensure label compliance requires a "walking pace," estimated to be approximately two mph. A two-man crew will conduct work, one ensuring that the applicator functions properly, and the other noting the route being followed, and anticipating obstacles and areas requiring the applicator to be shut down, including pedestrians or people out of doors. It is SCVC policy not to spray where people may receive direct exposures. Spraying begins at dusk, or sometimes a little before (sumithrin, the preferred insecticide for hand-held applications, degrades readily and rapidly in sunlight, and so such applications are less effective in daylight).

The hand-held routes are not performed with GPS equipment, and so the application route needs to be filed with GIS staff for mapping. Enhancement of SCVC equipment to allow GPS tracking of these sometimes intricate routes would be beneficial.

Setbacks from salt water are currently set at 100 feet. Setbacks from fresh water wetlands are set at 150 feet. These setbacks were negotiated with NYSDEC as a means of addressing perceived needs to regulate adulticide applications that fall within the 100 feet regulated Adjacent Area

buffer surrounding NYSDEC-mapped fresh water wetlands, and to similarly abide by label restrictions regarding applications directly to water. The specific modeling results associated with the risk assessment, and the risk assessment computation of ensuing impacts, provide a means to reconsider these bounds. SCVC should initiate discussions with NYSDEC staff at its earliest opportunity to determine of the setbacks need to be increased to provide more protection to the aquatic communities, or reduced to provide more complete control, especially in what may be key buffer area adult mosquito habitat.

On the mainland, essentially all vector control efforts are conducted using truck applications. Almost all air applications would require receiving an authorization to waive from fresh water wetlands regulations, which NYSDEC has not issued for non-health emergency adulticide efforts, pending completion of an EIS such as this one. SCVC pickup trucks are fitted with London Fog Model 18-20, ULV truck mounted aerosol generators that are equipped for adulticiding with an Adapco Monitor III GPS tracking and computer logger for ground-based adulticiding. The equipment is calibrated prior to the beginning of the season. Droplet spectrums are rechecked periodically. For mosquitoes such as *Oc. sollicitans* and *Ae. vexans*, the nozzle angle is set at 45 degrees to create a lower pesticide cloud. Should applications for canopy-dwelling mosquitoes (such as *Cx. pipiens* and *Cs. melanura*) be desired, the angle of the nozzle will be increased to 60 degrees from horizontal. Determinations regarding the target species will be made either by the SCVC Superintendent (for vector control applications) or the Commissioner of SCDHS in consultation with SCDHS and SCVC staff.

Maps of the target area will be generated by GIS prior to the application crew leaving SCVC offices. The maps will have no-spray lines, setback boundaries, and buffers surrounding other areas of concern clearly marked with strong colors to ensure the notations are discernable within the truck at night. SCVC tries to be sensitive for individual community needs. For example, spraying in Westhampton Beach was rerouted to avoid exposure for worshippers walking to synagogue one Friday.

The operation requires two people. One will operate the truck and application machinery. The other will be responsible for route maintenance and avoidance of obstacles, including timely

warning of pedestrians or people in yards (it is SCVC policy not to spray near people in the outdoors).

Spraying usually will begin at dusk, or sometimes a little later, and will continue for several hours to complete the route. This is for several reasons:

- Resmethrin, the Long-Term Plan preferred insecticide, including for truck applications, degrades rapidly under daylight conditions, and so efficacy would be lost through daylight applications.
- Most mosquito species, especially *Ae. vexans* and *Oc. sollicitans*, are most active at that time.
- Waiting for dark tends to minimize pedestrians and other outside venturers.

Pre-dawn applications target the same mosquito species, but often would be conducted at temperatures that are too low to meet operational requirements. Thus, it is proposed that almost all applications occur in the evening. Mosquitoes active later in the night, such as *Cx. pipiens* and *Cs. melanura*, could be targeted by having the application start several hours later (around 10 pm).

The vehicle must be moving at least seven mph for the sprayer to operate (that allows for proper dispersion of the spray cloud), and will cease operations if 20 mph is exceeded. The target speed is 10 mph. The sprayer is computerized, and so will calculate the release rate necessary to meet label limits. The sprayer also generates a GIS map of the route it followed, including on/off sites. It calculates the amount of pesticide applied. This information is downloaded on completion of the application, and is verified by the field crew prior to finalization by data management staff.

Setbacks from salt water are currently set at 100 feet. Setbacks from fresh water wetlands are set at 150 feet. SCVC will discuss the utility of these limits with NYSDEC in light of the risk assessment modeling and ecological risk calculations.

Some of the ground-based application events are under Health Emergency conditions. For those events, SCVC has almost always received an Emergency Authorization waiving certain fresh water wetlands restrictions, and need not abide by the voluntarily assumed setbacks for either fresh or salt water. As a practical matter, setbacks often ensue in any case due to the relationship between roads and waterways (roads seldom follow waterways without a buffer of some kind, and very often a residential lot is a very substantial buffer that exceeds 100 or 150 feet in depth). In addition, SCVC voluntarily adheres to measures requested by NYSDEC to limit environmental impacts, even when not required to by law, provided that can be done without compromising effectiveness. For Health Emergency applications, no-spray list restrictions need not apply, if waived by the Commissioner of SCDHS. Although it is not required by law, SCVC attempts to contact no-spray list members in an area targeted for an emergency treatment, in order to allow these individuals to take protective measure such as staying indoors or temporarily relocating during the spray event, if they so choose.

Aerial applications are almost always under Health Emergency conditions. This is because it is generally impossible to set helicopter swaths to abide by the NYSDEC setbacks, and because many vector control application events can be more limited in area than those conducted with a focus on addressing arbovirus presence.

The area selected for treatment is defined differently for each application mode.

- Hand held applications (strictly on Fire Island) cover the entire residential area in each community, excepting housing in buffers (for wetlands, open-water, and no-spray addresses), and the specific addresses on the no-spray list.

- The general area for a truck application for vector control purposes is generally defined by the locus of complaints. Complaints, while not sufficient to cause an adulticide application, are the most efficient means of defining areas with higher mosquito biting rates. Once a general area of interest has been defined, the application area is refined by including modifiers such as mandatory and voluntary setbacks (such as those around wetlands, open water, and no-spray list members), no-spray list addresses, environmentally-sensitive areas, farms, and other areas that should not be treated. The area road network also factors into the application area determination. This is because

issues such as large distances between streets, so that the application will not cover contiguous areas and so be less effective, may determine areas that it is not worthwhile to apply pesticides over. The tentative application determination is reviewed with SCDHS (typically, the ABDL director) for concurrence, and is used as a basis for public noticing. Application areas may continue to be refined until just before the run begins, although early determinations have the benefit of resulting in better route maps for the applicators.

- Health Emergency application areas are determined by SCDHS staff in consultation with SCVC. A focus of the determination is the extent of viral presence. The area to be treated also is set based on assumptions regarding the ranges of the potential human vectors. Complaints are sometimes referenced, as these can help identify areas where bridge vectors are especially active. Consultations with FINS, if required, can further define the application area. NYSDEC is routinely involved in the application area determination because there will generally need to be an Emergency Authorization which waives certain NYSDEC Freshwater Wetlands regulations. Practical considerations that need to be addressed regarding the capabilities of the helicopter that will apply the pesticides usually lead to a final application area determination. The practical considerations include (but are not limited to) the amount of pesticide that can be loaded onto the aircraft, the area that can be covered, and the geometry associated with making turns and applying pesticide in swaths. With the Adapco Wingman system operating, the actual final route followed by the aircraft will be determined in air, due to real-time feedback from the model, based on area weather observations and projected placement of the released pesticide. The Wingman model may also prove to be useful in developing efficient application area determinations.

The County uses a helicopter for aerial applications. It is a 3,200 lb. aircraft with an 18 foot six inch radius rotor operated by North Fork Helicopters, Ltd., of Cutchogue. The helicopter is fitted with two Beecomist nozzles nine feet from the centerline, oriented straight back. They have a flow rate of 25.2 oz/min. Prior to 2005, the applications means was by 300 foot swath released from 75 feet to 150 feet above the canopy at 70 mph. Modeling results indicated that off-target drift could be minimized by applying a 600 foot swath at 35 mph. It has been subsequently determined that in most situations, it will not be possible to slow the helicopter to

35 MPH for flight safety reasons. In addition, concerns were raised that slower speeds could increase droplet deposition, which could lead to greater non-target impacts. Instead, off-site drift will be reduced through the use of the Adapco Wingman system. Because the aerosols are intended to be composed of droplets so small they tend to remain suspended (they are brought to the ground more by turbulence than gravitational effects), drift caused by winds sometimes means the maximum pesticide concentrations do not occur in the center of the target area. This can be addressed through dispersion modeling, and leads to purposeful upwind offsets to bring the pesticide fully into the target area. To optimize this process, SCVC has acquired a state-of-the-art in-aircraft navigational-modeling system, produced by Adapco (the Wingman system). This system provides instantaneous course corrections to the pilot based on real time ground and balloon weather information generated in (or near to) the application zone.

The Adapco Wingman system includes settings that ensure the release of the pesticide will meet the specified rate. By directing the aircraft path so as to optimize pesticide delivery, it ensures that the minimal amount of pesticide to achieve the stated aims of the application is used. This will help the County meet its goals of pesticide reduction while still meeting its requirements for human health protection. The Adapco system is a proven (although cutting edge) technology, and has been shown to be effective and accurate in testing in other jurisdictions across the country.

The general flight pattern will be set with the pilot at the application area prior to loading pesticides into the helicopter, although the final route will depend on the on-board modeling output. The Adapco system, similar to the GPS guidance system in use at this time, will produce flight paths with on/off markings, and compute the amount of pesticide applied. The Adapco Wingman system ground module can also be used as a means of setting the proposed application area by forecasting an optimal swath pattern, given estimated weather. The timing of application events will follow those set for truck applications, above.

7.7 Resistance Concerns

All pesticide uses have an inherent risk of generating resistance in the target species. Resistance arises by selecting for individuals that are less susceptible to the pesticide being used. Applications that are not powerful enough are dangerous, because they will kill all of the most

susceptible individuals while allowing those with less susceptibility to provide the next generation.

Resistance is thus minimized by using appropriately high enough concentrations of pesticide. Resistance can also be minimized by alternating pesticides applied in order to reduce the potential of repeated use of only one formulation to select against that formulation. The probability of a mosquito being less sensitive to two different insecticides is reduced in comparison to the chances of being less sensitive to one, especially if they have different modes of action.

The formulators of the Long-Term Plan believe that the Caged Fish experiment justifies a reliance on resmethrin as an adulticide. Sampling associated with the experiment showed that the compound degraded extremely quickly. This means that it is extremely unlikely for it to have any environmental or human health impacts. It is not known if other modern adulticides degrade as quickly.

Reliance on one compound does raise resistance concerns. These are mitigated by the few adulticide applications made by SCVC over the course of a year, and by the small area impacted by adulticide events. This allows for a great many adult mosquitoes to reach maturity without contact with resmethrin. These mosquitoes will serve as a reservoir of genes to ensure that resistance does not become a dominant trait in Suffolk County mosquito populations.

However, this informal check on resistance is not sufficient. Therefore, SCVC should develop an improved resistance monitoring program. This kind of work is very specialized, and needs to be exceedingly precise and refined. This is because learning that the County has developed a sizable population of resistant mosquitoes would mean that it would be difficult to implement measures to relax selection and allow the return of susceptible mosquitoes. Good resistance monitoring determines if a problem is developing, and allows actions to be taken so that all pesticide tools can continue to be effective in achieving desired ends. New Jersey has an especially sophisticated program facilitated by Rutgers University Mosquito Research and Control Unit, and it is recommended that the County enter into a program with that group. The larger mosquito management companies (such as Clarke Mosquito Control) also offer such services, and could serve as alternate service providers.

7.8 Efficacy and Efficacy Testing

Adulticides are generally very effective. Indeed, the primary complaint about adulticides is a claim that they have negative impacts on too many unintended organisms. Under the best application conditions, it has been clearly demonstrated that all of the adulticides under consideration eliminate between 90 and 99 percent of mosquitoes within the treatment area (one to two orders of magnitude population reduction.

However, every insecticide application can be affected by a myriad of factors, including too much or too little dispersion due to weather, blockage of the insecticide from the target mosquitoes by foliage or buildings, or simple human error. Professional care and conduct address the latter sources of error in most instances, but sometimes natural conditions cannot be overcome, and the application fails to achieve its desired end.

In order to explicitly validate the County's adulticide program, the County should perform efficacy tests in association with every adulticide application, perhaps excepting those conducted on Fire Island in the communities, due to logistical difficulties. Two baited CDC traps would be set prior to every application, one in a control area, and one in the middle of the target zone. The mosquito problem confirmatory samples from the night before would then be compared to samples from the night after. Adjustments to the data sets would be made based on the control site results. The focus of the results would be on reductions in numbers of mosquitoes, and, when a health emergency has been declared, reductions in the parity and infection rates for the target species.

It must be understood that some mosquito species have a very quick generation time when the weather is warm and conditions are right; other mosquitoes are capable of migrating miles from their breeding sites in search of blood meals. So it is also possible to have an application of pesticides that kills the adults in a particular area, but still have a mosquito problem soon after that application as others migrate in or develop into adulthood. Therefore, because there is a lag from when the mosquitoes may or may not have been killed by the pesticides, and when efficacy data are collected, negative results for efficacy testing do not necessarily indicate that the pesticides did not succeed in killing mosquitoes in the target area. Testing within one night of the application tends to be a fairly good measure of efficacy, nonetheless.

SCVC also maintains a colony of *Cx. pipiens* in the laboratory. These mosquitoes are more usually used for laboratory investigations of such issues as pesticide effectiveness. However, mosquitoes can be put into cages, and set outside at appropriate or important sites to document adulticide application effectiveness. The results are generally recorded as the percent of exposed mosquitoes that succumb over a two or three hour interval. There are various technical issues associated with such studies, but nonetheless they are the general industry standard for assessing adulticide application effectiveness. This is because (for one) there is little chance that changes in weather immediately after the application will influence the mortality rate of the caged mosquitoes, whereas this is a common complication with trap tests. Caged mosquito testing is much more labor intensive than trap tests. The information generated by cage testing only bears on the immediate effectiveness of the application, and so is either very specific to the application, or is limited to the immediate time frame of the application (depending on one's point of view). Additionally, trap data have applicability for other aspects of mosquito control work. In sum, SCVC should conduct relatively few cage tests in any seasons (one or two are likely to be standard).

Each aerial application efficacy result set should be released within a week or so of the application. Results should also be released on an annual basis for the program as a whole. The individual events could be discussed in detail at that time.

8. Administration

8.1 Organization

SCVC works closely with SCDHS to ensure ongoing health related surveillance input for SCVC decisions are made. SCDHS operates the ABDL at the Yaphank facility and is also responsible for medical surveillance, environmental monitoring, and community outreach and public education, while the SCVC concentrates its efforts on mosquito control. An additional cooperative relationship exists between SCVC and SCDHS and NYSDOH to alert the County of statewide occurrences of WNV and EEE.

In the future, it is recommended that SCVC concentrate its resources on surveillance activities that involve assessing the population density and distribution of larval and adult vectors, while SCDHS continues to monitor and locate disease activity in mosquitoes and sentinel animals such as birds. Mosquito population surveillance (through New Jersey traps, larvae sampling, complaints, special traps set in problem areas, etc.) is intimately associated with the control operation and should be funded by SCDPW and be primarily a SCVC responsibility. While both SCVC and the ABDL will continue to be involved with mosquito surveillance, SCVC surveillance staff should be organized as a work unit that collects and receives New Jersey trap collections, larval samples from the SCVC crews, and conducts special larval and adult collections designed to manage the control effort. The ABDL will employ more technically demanding sampling methods, such as cold chain, which involves keeping specimens cold to prevent viral degradation.

In order to implement the recommendations of this Long-Term Plan, it is expected that significant additional resources of both personnel and equipment will be approved by the County to improve vector control practices in accordance with the findings of this study. SCDPW and SCDHS have prepared specific proposals detailing the number and titles of new personnel required to implement this program. The actual creation and filling of these proposed positions, however, is dependent upon the County budget process.

Administration

The Vector Control Superintendent will be responsible for the overall administrative supervision and the supervision of mosquito management actions. Because of intense regulatory scrutiny,

the Superintendent will particularly administer aerial larvicide and all adulticiding operations. There will be expanded responsibilities for this position as the operations of SCVC become more technically complex. New oversight by various committees and cooperative outreach to towns and other government agencies will also increase the workload. The expanded mandate with respect to wetlands management will be an additional set of new responsibilities. The end of the Long-Term Plan project should facilitate the time and efforts necessary to deal with these new expanded duties. The existing duties of this position are:

- Provides overall administrative supervision and operational oversight of mosquito management actions and wetlands projects.
- Coordinates activities of units within the Division.
- Evaluates Division operations and effectiveness.
- Coordinates with other County Departments, especially SCDHS, the ABDL, and other government agencies.
- Interacts with public.
- Interacts with professional associations and other mosquito control agencies to ensure the program operates to current standards, and stays abreast of developments in the field.
- Serves as technical resource for staff.
- Responsible for Division response to litigation, including coordination with counsel, testimony, and other legal issues.
- Oversees aerial larviciding based on surveillance reports.
- Issues public notice of aerial larviciding.
- Oversees adulticiding and makes determination of need based on a range of surveillance and other factors.
- Directs aerial adulticiding operations.

- Issues public notices for adulticiding.
- Designs water management projects.
- Selects water management equipment.
- Obtains Article 15 Aquatic Pesticide permits and Fresh Water Wetland permits.
- Serves on County Pest Management Committee.
- Prepares Annual Plan of Work and budget. It is anticipated that the success of the Long-Term Plan may reduce responsibilities in this area somewhat, as future Plans of Work may cite the Long-Term Plan extensively instead of preparing de novo material each year.
- Supervises the SCVC surveillance effort to ensure that all control is surveillance-driven.

Specific new responsibilities include:

- Oversee the preparation of the Annual Wetlands Strategy Plan.
- Assist in the preparation of annual efficacy reports.
- Oversee the implementation of the Long Term Plan.
- Oversee preparation of the triennial Long-Term Plan compliance report.

SCVC will use Long-Term Plan to assist in the preparation of Annual Plan of Work. The Plan of Work is a written description of SCVC's purpose, history, current operations, and goals for the following year and the future. The Plan of Work is prepared by the Superintendent and submitted to the Legislature in October for approval in November. The Legislature approves SCVC Plan of Work each November as part of the County Budget.

General administrative support for SCVC will come from the SCDPW Administration and will include duties such as payroll, purchasing, etc. This unit will take service requests and handle other public contact, and support litigation response by providing files and other pertinent

information. Given the increased activities proposed for SCVC, there will be a need for additional administrative staffing. The existing staff includes one Purchasing Technician and one Clerk Typist. The existing duties of the Purchasing Technician and Clerk Typist are:

- Taking service requests and other public contact.
- Sending out routine notices.
- Various administrative tasks, such as purchasing, personnel, vehicle management and other administrative details.

No new duties are proposed. However, the greatly increased activity will require additional staff. Under the Long-Term Plan, there will be more time consuming tasks of public outreach and contact, and the new work units will add to the administrative support workload for which budgetary support will be required.

Technical Services and Compliance

The Technical Services and Compliance unit will coordinate and approve all data collected by SCVC, while providing technical support for the other units. This unit will oversee all SCVC activities for environmental compliance and ensure that all required reports are prepared. This unit will also be responsible for some of the technically demanding tasks of SCVC, such as equipment calibration and adulticiding. All data collected by SCVC must be made immediately available to the ABDL. To accomplish this, SCVC will task its Programmer/Analyst and other staff with developing improved data systems to facilitate rapid collection and dissemination of adult and larval data over the network. Access to these data will be given to the ABDL.

There is a need for a highly trained and experienced Principal Environmental Analyst to handle these tasks and oversee day-to-day operations, since it is not possible for the Superintendent to perform these tasks and also handle administrative duties. Given the high visibility of the program, the extensive set of laws and regulations that pertain to it, and the high likelihood of continuing litigation, maintaining proper data systems and oversight to maintain and document compliance is a critical activity. At the current time, the Technical Services and Compliance unit consists of:

- one Principal Environmental Analyst
- one Programmer/Analyst
- one Biologist (this position will be moved to a new Natural Resources Unit, if created)

The existing duties of this unit are:

- Overseeing control operations (normally limited to stand-in for Superintendent during his absence).
- Supervising wetlands projects.
- Obtaining water management permits and conducting other activities to maintain environmental compliance.
- Preparing permit maps and other materials (to be transferred to Natural Resources).
- Overseeing heavy equipment unit (to be transferred to Natural Resources).
- Assisting in viral surveillance (to be transferred to ABDL).
- Preparing maps and aerial photography for use by other units.
- Operating SCVC GIS.
- Developing GPS/GIS and adapting to SCVC needs.
- GPS mapping for water management (to be transferred to Natural Resources).
- GPS/GIS for larvicide application (to be transferred to Mosquito Surveillance and Control).
- Designing, operating and maintaining data systems to ensure relevant and required data is obtained and is available for analysis.
- Providing equipment calibration and documentation.

- GPS/GIS for ground adulticiding.
- Gathering information from field and preparing State pesticide reports.
- No-Spray list maintenance, management, and compliance.
- Complying with Freedom of Information Law and discovery requests for information.
- Alternate to Superintendent for public notices.
- Alternate to Superintendent for aerial larvicide applications.
- Alternate to Superintendent for adulticide applications.
- Technical support for litigation.
- Interacting with Long-Term Plan
- Special sampling of problem areas and other needs (to be transferred to Mosquito Surveillance and Control)

A GIS specialist will be required to: receive data from field crews and integrate it into the overall system and to assist the Mosquito Surveillance and Control and Natural Resource units in acquiring GIS/GPS data and provides information for reports.

The proposed new duties for the Technical Services and Compliance unit that will require increased staffing are:

- Operate the ADAPCO air system.
- Prepare operational summaries for reports and public outreach.
- Develop priority lists for water management actions.
- Act as technical resource for Mosquito Surveillance and Control and Natural Resource staff.

- Act as staff for wetlands committees.
- Develop data systems for monitoring and compliance.
- Web Master for improved public outreach.
- Outreach to towns, other governmental agencies, and non-governmental organizations.

Mosquito Surveillance and Control

The Mosquito Surveillance and Control unit will be reorganized and upgraded to process more information to guide control decisions and evaluate the control efforts. This unit will guide the larval control program. It will determine the need for adult control and refer that task to Technical Services and the Superintendent for action. Existing staffing is not sufficient to provide trapping data in all locations where adulticiding occurs. Greater follow-up and a new quality control effort are to be implemented will also require additional resources. Similarly, as more information is to be provided to the public to support the program, this information must be compiled and put in a useful format. This information would also be used in determining the need for additional control if pathogens are present. This unit could assist the ABDL in viral surveillance during peak times and emergencies, but these duties would normally be transferred out of SCVC to the ABDL. The information gathered would also be used for compliance reports. ABDL data should be made available to SCVC, to the extent permitted by medical confidentiality laws. Collection of field samples should be coordinated between SCVC and the ABDL to avoid duplication of effort.

The Mosquito Surveillance and Control unit currently consists of:

- one Vector Control Supervisor
- one Vector Control Aide
- one Laboratory Technician (vacant)
- one Auto Equipment Operator (seasonal)

The existing duties of this unit are:

- Operate New Jersey trap network, count samples, and enter data.
- Identify larvae collected by field crews.
- Assist the ABDL in virus surveillance (to be transferred to upgraded ABDL except for emergencies).
- Assist with special studies.
- Maintain a mosquito colony.

The proposed new duties that will require additional personnel for the Mosquito Surveillance and Control unit are:

- Overall responsibility for assessment of vector mosquito populations, determining the need for vector control, directing control efforts and evaluating the effectiveness of control measures.
- Operate an expanded network of New Jersey light traps, analyze samples, enter data into appropriate systems and analyze and interpret results
- Review and analyze service request data to identify problem areas.
- Refer areas requiring adulticiding to Superintendent and Technical Services and Compliance.
- GPS/GIS for larvicide application (transferred from Technical Services and Compliance).
- Conduct surveys of larval abundance, distribution, and species composition using samples gathered by field crews, and conduct supplemental sampling.
- Carry out special adult or larval collections to investigate problems.
- Carry out special surveys of problem larval habitats for species such as *Cq. perturbans*.
- QA/QC evaluation of control efforts using special trapping or other sampling measures.

- Use service request data to ensure problem areas are identified and addressed.
- Provide data on sampling and applications for compliance reports.
- During winter, evaluation of program effectiveness and other data analysis.
- Conduct special studies on new materials and/or innovative control measures.
- Conduct biocontrol using fish or other predators.
- Identify priority areas for water management measures.

Natural Resources

The Natural Resources unit, which is a newly proposed unit, will be responsible for the implementation of an expanded, far more sophisticated, progressive water management program. This will require more attention to natural resource issues and more detailed project planning, documentation, and evaluation. In particular, even the most minor maintenance actions will require more documentation, and simple culvert replacements and upgrades will require engineering-level drawings. Survey skills will be necessary, at a minimum, and complex projects may require sophisticated engineering design. Engineering skill may also be required for SCVC input into USEPA Phase II Stormwater Management actions. Even if other agencies have available resources to perform project monitoring, SCVC will need to guide and evaluate these efforts. New personnel to staff this unit are needed for the following duties:

- Oversee collection of natural resource data for permitting, compliance and monitoring, and for supervision of wetlands projects.
- Assistance in surveillance and quality control during the summer months, especially evaluation of control measures, and to assist Superintendent with supervision of wetland projects.
- Design of water control structures, with a focus on determining the appropriate sizes of culverts and tidal channels. Preparation of project drawings, especially for more complex projects. Engineering design of more complex structures such as tide gates.

- Surveys of wetlands projects and water control structures and preparation of project drawings, e.g., drawings for standard structures such as replacement culverts.

Proposed duties of the entire unit are:

- Responsible for collection of natural resource and mosquito data for permitting, compliance, and monitoring, as well as wetlands stewardship activities.
- Design and engineer wetlands management projects.
- Provide all necessary project plans and supporting information for permitting and compliance.
- Supervise construction of wetlands projects.
- Document all water management activities and provide data for information systems.
- Conduct monitoring and assessment of County wetlands, including use of remote sensing.
- Provide information to allow setting priorities for water management.
- Assist landowners in monitoring efforts.
- Evaluate effects of water management activities.
- Conduct special studies for non-target effects.
- Act as staff for wetlands committees.

Field Crew and Water Management

The Field Crew and Water Management unit will perform the daily technical tasks such as water management and pesticide application for SCVC. This unit will also conduct larval surveillance, assist with adult surveillance, and respond to service requests. Thus, this unit will represent the working component of the program, while serving as its sentinel.

Existing staffing for this unit is:

- five Vector Control Labor Crew Leaders
- one VC Supervisor (temporary for Fishers Island)
- two Temporary Labor Crew Leaders (Fishers Island)
- one Vector Control Aide
- three Construction Equipment Operators
- three Heavy Equipment Operators
- 18 Auto Equipment Operators (including two currently vacant positions)
- four Laborers (including one currently vacant position)

The proposed new duties that will require additional personnel for the Field Crew and Water Management unit are:

- Increase larval surveillance to assist with guiding control decisions.
- Increase reliance on larviciding and water management to reduce adulticiding.
- Increase control of breeding in stormwater structures, such as catch basins.

Arthropod-Borne Disease Laboratory (SCDHS)

The ABDL presently operates using a combination of SCDHS and SCVC staff to conduct viral and population surveillance. This practice creates a situation whereby the same staff members collect information related to the control aspect of the program as well as information for the disease aspect of the program. This results in programmatic competition for limited staff time. The ABDL and SCVC both need increased resources, and especially staff, to implement the draft management recommendations. Given the high priority of viral surveillance, resources are often not available to provide data and analysis directly related to the control program. In addition, the lines of supervision, control, and budget are complex and not conducive to optimal use of

resources. Under the proposed organization, the ABDL would be clearly tasked with viral surveillance and would control all resources needed to conduct that work. This would allow assignment of SCVC staff for activities critical to that unit, and relieve the ABDL of tasks more directly related to the control program than to disease surveillance. When the ABDL identifies viral activity, the information can be easily combined with that collected by SCVC to guide response measures. In fact, increased and more sophisticated surveillance by SCVC on vector populations should lead to a more targeted response to viral activity.

SCVC staff will manage its workload to allow it to assist with viral surveillance, if needed, during the peak viral season (August and early September). However, peak viral season historically has coincided with the times when the demands on SCVC staff associated with the complexities involved in adulticide planning, permitting, and follow-up have also peaked. If this seasonal pattern continues under the Long-Term Plan, it would limit SCVC's ability to provide assistance. ABDL staffing levels should not be based on an assumption that SCVC staff will be available for all peak viral surveillance workloads. During times of a declared public health threat, all surveillance and control resources will be controlled by SCDHS, as outlined in the County Charter. High priority viral sampling may have to take priority over other surveillance. SCDHS will be required, of course, to continue to ensure that all aspects of the Long-Term Plan are complied with, to the maximum extent practical.

Staff from this unit will report to SCVC on a daily basis, but may report to the ABDL during emergencies.

The existing staff of the ABDL and their corresponding duties are:

- One Laboratory Director: Responsible for overall administration and supervision of laboratory.
- Two Entomologists: Perform infectious agents surveillance and testing.
- One Biologist: Performs dead bird testing using the RAMP system as well as assist with infectious agent surveillance and testing.
- One Program Aide: This staff member serves as the Health Safety Officer, performs budgetary tasks, and obtains the necessary permits for laboratory function.

As a supplement to the existing positions listed above, significant new staffing resources will be needed to implement the proposed management plan recommendations for the ABDL. The department has a specific proposal for consideration during the county budget process. All sampling, testing, and analysis for the presence and distribution of mosquito-borne pathogens should be transferred to a stand-alone ABDL with full capabilities to conduct this work. Staffing level and other resources, such as vehicles, must be sufficient to provide this capability. The level of resources will depend to some extent on how much testing will be done in-house. Data from this effort would be combined with SCVC data on vector populations, plus human surveillance conducted by SCDHS, to assess the risk of mosquito-borne disease and to determine if measures beyond general vector control (such as special adulticiding) are required. Resource sharing between SCVC and ABDL is possible and necessary. Examples include deploying and recovering traps. There are, nonetheless, advantages to a more formal division of labor between SCVC and the ABDL. The current situation has the same staff collecting information related directly to control and information for virus survey. This can lead to competition for limited staff time. Since virus sampling has the highest priority, data collection related to the need for and the evaluation of control efforts may not be completed. The best way to ensure more data is collected to assess the need for control and to evaluate any control efforts, while not decreasing pathogen sampling, is to provide the resources that allow the two programs to operate independently.

In summary:

- It makes organizational sense for SCVC to collect and manage the data it needs for its day-to-day control operation.
- It makes organizational sense for SCDHS to survey for human pathogens.
- Most of SCVC's effort is preventative and conducted based on the abundance and distribution of vectors, rather than in direct response to pathogens, and so is conducted prior to and independent of the detection of pathogens.

- SCVC's sampling needs are directed mostly toward those areas where mosquitoes are most abundant, while the ABDL is most concerned with determining where pathogens may be present.

- Vector sampling is time-critical, in that daily control decisions depend on it.

- The samples collected for monitoring purposes by SCVC do not require being kept in cold storage after collection, as those collected by the ABDL for viral detection do.

- A division of labor between the sampling programs allows each one to operate in a manner that optimizes its efforts.

The current level of coordination between the ABDL and SCVC regarding adulticide decisions when there is no declared health threat appears adequate. The standard e-mail notices for the adulticide operations should include a brief description of the surveillance indicators for the operation, a practice that was begun in 2005. During a declared health threat, adulticide decisions are controlled by SCDHS as required by the County Charter. It has been standard practice at these times for SCDHS to delegate control decisions based on mosquito population levels to the SCVC Superintendent. Decisions regarding applications in direct response to viral findings and human disease risk have been made by SCDHS, with technical input from SCVC.

The County currently has a capital project in progress to upgrade SCVC facilities and the ABDL. Upgrading the laboratory will provide it with the BSL-3 certification required to become fully autonomous. Obtaining this certification would allow samples to be processed in-house, decreasing the amount of time required to obtained results significantly. The BSL-3 certification would also provide the ABDL with the ability to test samples for all types of mosquito-borne viruses, such as EEE. Under the current scenario, sending samples to Albany is a necessity because the state laboratory tests for all types of mosquito-borne viruses, such as EEE and St. Louis encephalitis, while the Taqman and RAMP methods only detect WNV. Testing for all types of mosquito-borne viruses ensures that field detection systems and laboratory detection systems are working, and that unexpected arboviruses do not pass unnoticed. SCVC and the ABDL should share lab facilities, wherever these facilities ultimately are built, to avoid duplication and facilitate coordination.

8.2 Professional Education

Continuing education provides professional staff with the opportunity to gather information on current and novel mosquito control techniques. Professional education for mosquito control workers includes:

- pesticide training programs
- equipment training programs
- computer software training programs
- field techniques training programs
- short courses in mosquito identification and control
- "Right to Know" training for hazardous substances
- attendance at state, regional and national mosquito control conferences

Pesticide applicators are required to obtain professional applicator certification and to maintain that certification. Many of the County's applicators are certified both as Public Health Pest Control applicators (Category 8 of the NYSDEC strata) and Aquatic Insect and Miscellaneous Aquatic Organism Control applicators (Category 5B). Certification requirements for both include obtaining 18 credits of continuing education over a six year period, and those with dual certification must obtain credits in both areas. The NYSDEC website (http://www.dec.state.ny.us/website/dshm/pesticid/appman.htm#certification) contains a great deal more information regarding certification and recertification requirements. These courses tend to be offered locally, often at Stony Brook University.

Formal courses offered in the immediate area that would be of value to SCVC and ABDL personnel include species identification short courses taught at both Rutgers and Cornell. Travel restrictions make attendance at these courses difficult. Although Cornell is located in-state, the distance from the County means overnight stays are a necessity. The Rutgers courses can be commuted to, but constitute out-of-state travel, which is currently restricted by County policy.

Attending general state, regional, and national mosquito control meetings benefits staff productivity and presents networking opportunities, allowing SCVC and ABDL staff to objectively compare the performance and quality of the County's program to that of others. The formal presentations offer obvious means for program evaluation and improvement. Underappreciated, perhaps, are the benefits associated with mingling with other professionals in the field, discussing mutual problems, and sharing potential solutions with one another. While journals and electronic sharing of presentation materials can be sufficient so as to minimize some of the advantages for attendance at formal presentations, informal networking is difficult to simulate without face-to-face contact. For this reason, more frequent attendance at such meetings by County personnel is a strong recommendation of this Long-Term Plan. Information from these various sources can then be incorporated into the existing program, directly upgrading quality. County policies regarding travel out-of-state by employees needs to be relaxed to ensure that professional staff retains its professional qualifications and keeps the program operating at the highest standards.

Specifically, the productivity of SCVC staff, ABDL staff and the existing mosquito control program would benefit by allowing additional travel. Two regional meetings should be attended by two additional professional staff, such as an entomologist and biologist. There should be regular participation in additional regional (Northeastern Mosquito Control Association, Mid-Atlantic Mosquito Control Association, and New Jersey Mosquito Control Association, as examples) and national meetings (CDC annual WNV conference, AMCA national and Washington meetings, and the Society of Vector Ecologists, as examples) by the Superintendent and ABDL Director. Suffolk County should also participate in the Associated Executives of Mosquito Control in New Jersey, an organization of superintendents and other key mosquito control officials that meet on a monthly basis. The Associated Executives provides a forum for officials with similar issues and problems to share information. It helps prevent "re-inventing the wheel" by more than one agency, saving time and money for all concerned. Technical staff should also attend professional training offered at Rutgers and/or Cornell in mosquito biology and identification to improve their mosquito identification and sampling skills. Such training will be especially valuable for field technicians responsible for retrieving traps from distant locations, such as the north shore, and utilizing proposed identification stations.

9. Technology Assessment

Mosquito and wetlands management require the use of sophisticated machinery and equipment of various kinds. This discussion is limited, as it is clear that technological innovation will make the current equipment and the recommendations here obsolete in fairly short order. Nonetheless, some broad guidance is offered in order to ensure the program maintains its current sophistication, and to provide certain necessary improvements.

9.1 Data Management

Mosquito management does not require sophisticated, statistically-based data analysis, for the most part. Certain environmental monitoring data sets (generally associated with wetlands management projects) may, but most will require the same kinds of simple trend analysis that the mosquito data calls for. However, especially for mosquito management, geographical trend analysis appears to be key. This calls for a reliance on GIS data management.

In addition, "once in" data entry is much preferable to re-entry or downloads/translations. Laboratory data entry can be streamlined by installing system desk tops at the analysis spaces. Field data entry is another issue. The customized VCMS system currently used does have advantageous remote entry capabilities, and is well-suited to the kinds of information generally produced by the surveillance and treatment programs. However, it does not interface well or easily with other programs. Although technical support is good, this does not cover the effort required to translate data into GIS formats. It is suggested that compatibility be a goal, as the lessons of the Microsoft era appear to be to that the trade-off in forsaking optimal programs for suboptimal is acceptable due to the benefits of interchangeability.

A fair degree of training will be required to ensure all necessary personnel are competent with the GIS software. However, this appears to be in accord with general County government policies.

To support GIS data entry, GPS needs to be universally available, in all equipment, and for all personnel.

9.2 Wetlands Equipment Needs

Compared to programs such as those found in New Jersey, Suffolk County does not need the extremely large, specialized marsh excavation machinery commonly used in jurisdictions that have very large wetlands that may exceed 1,000 acres in size. These machines, while highly productive, are difficult to move from one wetland to another without disassembly, which is a drawback in Suffolk County, with its numerous, smaller wetlands. On the other hand, many projects in Suffolk County will be larger than those addressed in Connecticut, where mechanized equipment is relatively sparse. Fortunately, Suffolk County already possesses an array of specialized marsh equipment that is well suited to local conditions. This equipment includes machines mounted on low ground pressure (less than two pounds per square inch) or amphibious tracks. When properly handled, these machines can operate on the soft terrain of Suffolk's wetlands with little or no adverse effects on sensitive vegetation. Current equipment can accomplish all the necessary tasks required to implement the Wetland Management Plan BMPs. However, additional equipment would speed up the pace of implementation. There is a particular need for personnel carriers to transport crews and supplies and for a long reach excavator to facilitate pond construction. In addition, at least one excavator should be fitted with a rotary excavating bucket to facilitate the sculpting of tidal channels and ponds.

9.3 Adulticiding Issues

Major choices for the County in terms of technologies for adulticiding include the means of application, choices between application platforms, and the use of models to support decision making and the proper application of the pesticides.

There is no question that ULV treatments are the application means of preference at this time. Suspending fine droplets of insecticide in the air allows for the mosquitoes to fly into the droplets and receive fatal doses. It is efficient, because the use of inert substances is minimized, and so aircraft with load considerations or trucks with volume limits can easily carry sufficient product to meet application requirements. Concentrating the pesticide also minimizes exposure to compounds that may not have received the same degree of regulatory scrutiny that the active ingredients did.

Thermal fogging, however, also has a place with SCVC for special situations. Fogging is essential if tire piles become a problem, as it can penetrate into the mass of the tires (where the ULV cloud would not). Fogging has also been suggested as a way of treating adult mosquitoes in stormwater systems (although Los Angeles has also developed a ULV delivery system).

SCVC currently uses a helicopter for aerial pesticide applications. For the near future, it is likely that aerial applications of pesticides will still be a program need. If progressive water management is successful, the incidence of aerial larviciding may fall dramatically, perhaps to the point where SCVC will seek a different delivery mode for infrequent applications to large marshes. For the near future, however, larviciding will continue; and there is no real prospect of avoiding adulticiding for health emergencies. Airplanes, a mainstay for many US programs, are not needed, and would be unwieldy for operations, given the relatively small areas treated by the County.

It also seems appropriate to continue to lease services from a private operator rather than to purchase a helicopter for County use. An analysis published for New Jersey suggests that the magnitude of the current program is at the cusp where helicopter purchase may be more economical. However, it is envisioned that larvicide applications, for one, will be greatly reduced within the operational life-span of a helicopter. A potential to share an aircraft with Nassau County has some attractiveness, as between the two Counties the need for the aircraft could certainly be justified. Both Counties have helicopters for other purposes, and so have appropriate maintenance and upkeep capabilities. However, if a major virus outbreak should occur, a shared platform could lead to disputes regarding first-use or other resource allocations. Shared resources between separate programs is often most appreciated from a theoretical vantage point, as there are many practical difficulties that often can be resolved suboptimally.

It seems clear that there is a continuing place for truck adulticide application capabilities, as well. Truck applications can be more limited in area than air applications. There have been some concerns regarding the ability of truck applications to evenly distribute pesticide due to the presence of obstacles that can interfere with dispersion, as does not happen with aerial releases. In addition, because the release point of the material is close to the ground, they represent a higher potential human exposure than an aerial application, where the aerosol is well dispersed

prior to reaching ground level. This potential is mitigated by County policies that avoid exposure to people outside of their homes at the time of application. Truck treatments can be more effective than aerial applications where the canopy is closed. They may also be more effective in denser woodlands for impacting canopy-dwelling mosquitoes. Where the canopy is more open, the generally greater dispersion of aerial applications makes them very effective at accessing the canopy from above and below.

SCVC has purchased an Adapco Wingman product to help guide aerial applications to optimize the pesticide dispersion pattern. SCVC has also obtained further calibration services from RTP Environmental through the Long-Term Plan to validate the model output. SCVC should be aware of future advances in mosquito application modeling, as this technology is relatively new, and so has the potential to quickly change in potentially major ways. SCVC should also carefully evaluate the utility of real time course adjustments, as there have been some concerns raised that the stable atmospheric conditions under which many applications occur lead to large dispersion changes as the result of small weather variations. This may mean that small changes in wind speed or direction may indicate large changes in aircraft direction. Multiple course changes over an application area may not be optimal for either efficiency or overall pesticide application reasons.

9.4 Laboratory Issues

The County has to carefully analyze fiscal and operational justifications for the construction of a BSL-3 laboratory. There are several important reasons to create a local BSL-3 facility:

- It will allow for the ABDL to conduct all of the kinds of sample analyses that it needs to do to fulfill its mission

- Because a laboratory upgrade is required in any case, it is prudent for the County to build the kind of facility that will serve it well for decades, rather than “making do” with an inadequate facility purely to minimize costs for the short-term

- Security and worker safety issues probably will require a facility that is “BSL-2+” in any case, so it makes sense to explicitly move to BSL-3 for somewhat modest cost increases from BSL-2+ levels.

Currently there are BSL-3 Laboratories at Plum Island and Stony Brook University operating in Suffolk County. SCDHS attempted to contract with Stony Brook University, but significant costs and lack of mutually acceptable financial terms prevented a contractual agreement from occurring. The facility at Plum Island is under the auspices of the Federal Department of Homeland Security, and it has recently proposed the phase-out of the facility because of inefficient operations. There are significant issues, therefore, associated with both potential partners:

- financial with Stony Brook University;
- issues associated with access (ferry only) and the uncertain future for Plum Island.

It needs to be noted that these issues do not necessarily preclude success if future negotiations were to be undertaken, but do suggest the magnitude of those potential negotiations.

NYSDOH currently supplies the County with the testing information it would receive from its own BSL-3 facility. In previous years, due to budgetary limitations that limited the number of times the County could send samples to Albany, turn-around times on virus determinations was a public health issue. Quicker turn-around times (from 10 to 14 days to several days) reduce the impact of this. It should be understood that, at best, use of NYSDOH will result in turnaround times of at least several days, which would exceed turnaround times associated with use of a local facility. Turnaround times at such a local facility could potentially be consistently less than 24 hours.

The potential for resource limitations at the State facility is a concern that cannot be addressed. NYSDOH has (under the no-cost service) limited the number of samples that can be processed at particular times. This may be the key policy issue that determines the need for a Suffolk County facility. The County may be required to forego information necessary for public health decisions because of limitations on outside laboratory capacity. This is the most compelling point in favor of constructing a local BSL-3 facility since there is a significant need for rapid testing results to allow for swift formulation and direction of mosquito control strategies.

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10. Adaptive Management

10.1 Introduction

The Long-Term Plan is not intended to be static. This is for two basic reasons. One is that changes in disease occurrence, technology, or conditions may require changes to the Long-Term Plan as currently envisioned. It may be that the basic direction described here is still the means by which the County wishes to achieve its ends, but exact methods need refining. If that is the case, the Long-Term Plan does not need to be entirely reworked, but merely massaged to account for the changes.

Secondly, some parts of the Long-Term Plan forthrightly express that necessary information to complete the planning process was not yet available, or could not be compiled at this time. As that information becomes available, changes in or more complete descriptions of plans will be constructed.

10.2 Structures and Mechanisms

The basic structure of the Long-Term Plan process should remain in place. The Steering Committee would still have overall policy responsibility, except now it will be for implementation of the Long-Term Plan, as well as residual planning processes and the adaptive management steps that occur. The Steering Committee would receive technical advisement by the Technical Advisory Committee, and the Citizens Advisory Committee may also continue to function as an outlet and input device to the Long-Term Plan for concerned citizens and advocacy groups. In addition, on wetlands issues, the reconstituted Wetlands Subcommittee and Wetlands Stewardship Committee will serve as valuable adjuncts to provide information to the Steering Committee for its decisions. It is clear that some level of expanded staff support will be required to accommodate the continued coordination and organization of these units.

Basic planning will not be addressed by the consultant team, but rather will become a SCVC responsibility. SCVC will support this planning responsibility by actively seeking cooperative exchanges with Federal, State, and local agencies and governments, and by reaching out to other interested parties and advocates. SCVC will drive much of this interaction as it maintains its

own knowledge base through participation in continuing education and attendance at professional meetings, and membership in professional societies and groups, but will also remain open to the suggestions and requests by local experts and other interested parties.

The mechanisms by which the Long-Term Plan can be amended include:

- Changes referenced in the Annual Plan of Work. Each Plan of Work will be required, in order to meet SEQRA requirements, to comply with conditions and thresholds set in the Long-Term Plan. This does not prevent minor changes to the Long-Term Plan from being introduced through the Annual Plans of Work. Each Annual Plan of Work will be appended to the Long-Term Plan to make that mode of change explicit.

- The triennial Long-Term Plan Update. This report also provides a mechanism for adjusting plan Goals and Objectives, and determining if adjustments need to be made to specific areas of the Plan. Each one will also be appended to the Long-Term Plan.

The following outline is intended to provide a preliminary overview of issues which will be analyzed to form the basis of the triannual report. The outline includes indicators (where available) which will be used to measure success. The content and format of the triannual report will be contingent on Steering Committee and Wetlands Stewardship Committee input and approval, which will be sought at the early stages of report preparation.

1) Executive Summary
The Executive Summary will provide an overview of the following issues, which will be addressed in detail in subsequent report sections.

- Public health (viral surveillance, human disease)
- Vector control (pesticide usage, water management, surveillance, etc.)
- Education/outreach
- Wetlands Stewardship Program – Accomplishments and Plans
- Plan Updates and Amendments

2) Public Health

- Viral surveillance results
- Human health (cases and deaths from mosquito-borne diseases)

3) Vector Control Long-Term Plan Implementation
The report will integrate results from the Department of Public Works, Division of Vector Control and Department of Health Services, Division of Public Health.

A. Public Education and Outreach

Current Program:

- Recommend avoidance of the outdoors at dawn and dusk.
- Consider use of personal repellants (DEET, Bite Blocker, Picaridin, Oil of Eucalyptus).
- Maintain home environments that do not foster mosquito breeding.
- Distribute Publications such as "Fight the Bite" and "Dump the Water."
- Maintain County Web Site
  - Post spray events
  - Link to no spray list

Long-Term Plan Recommendations:

- Establish tire management education program to eliminate mosquito breeding habitat. Encourage other county departments and municipalities responsible for routine sanitation or maintenance activities to properly dispose of tires.
- Conduct farmer irrigation outreach-targeted education through Cornell Cooperative Extension.
- Encourage private storm water system maintenance.
- Conduct tailored outreach to municipal highway departments regarding stormwater structures as mosquito habitat.
- Emphasize personal responsibility for reducing impacts from mosquitoes (avoiding mosquitoes whenever possible, wearing long-sleeves and pants, and using repellents).
- Improved efficacy reporting. Results made available to the public via the web and annual reports.
- Post efficacy reports on the SCVC website. Reports will summarize the results of mosquito control efforts measured before, during and after aerial spray event.
- Maintain the Citizens Advisory Committee .
- Create a listserv for adulticide application notifications.
- Integrate new web site into existing county site.
- Revise public notice/guidance .
- Participation in "Mosquito Awareness Week."
- Targeting specific communities (recommended in DGEIS comment period).
- Focusing on educating school-aged children (recommended in DGEIS comment period).

Indicators of Success

- Degree to which current program and Long-Term Plan recommendations are implemented. Implementation will be quantified, where possible. E.g.:
  - Partnerships established with towns for tire management plans.
  - Public education workshops which have been conducted.
  - Brochures and fact sheets disseminated to public.
  - Number of efficacy reports posted.
  - Programs targeted at specific communities and school-aged children.

B. Scientific Surveillance

Current Program:

- Presence or absence of larvae
- Collect and process 10,000-12,000 larval and adult mosquito samples
- Collect and process approximately 75,000 mosquitoes for arbovirus surveillance
- Integration of Geographic Information System (GIS) and Global Positioning System (GPS) technology for surveillance information
- 27 permanent NJ traps; 80 CDC trap-nights per week.

Long-Term Plan Recommendations*:*

- Increase surveillance capabilities.
- In-house virus testing through the proposed BSL-3 laboratory project.
- Increase staff for surveillance for both SCVC and the ABDL.
- Increase permanent NJ trap network to 30.
- Increase CDC trapping to 105 trap-nights per week.
- Conduct quantitative mosquito assessment prior to EVERY adulticide event.
- Conduct post-spray efficacy monitoring.

Indicators of Success

- Degree to which current program and Long-Term Plan recommendations are implemented. E.g.:
  - Number of staff-days dedicated to surveillance.
  - Number of mosquito samples processed.
  - Number of CDC light traps deployed and NJ traps maintained.
  - Number of pre-adulticide mosquito counts.
  - Annual reports on surveillance analysis, including post-spray efficacy.

C. Source Reduction/Control

Current Program:

- Public education program (above).
- Response to citizen complaints.
- Catch basin and recharge basin control efforts.

Long-Term Plan Recommendations:

- Expand surveillance of catch basins from 10,000 to 40,000 inspections.
- Augment education component (County tire collection effort, private storm water management system outreach effort, increase interaction between SCVC and highway departments)

Indicators of Success

- Catch basins inspected.
- Records on response to complaints.

- Improve waste management and county departments tire management

D. Biocontrols

Current Program:
Mosquito fish, (Gambusia spp.)

Long-Term Plan Recommendations:
- Fathead minnows, native to the area (Pimephales promelas)
- Predacious Copepods

Indicators of Success
- Research alternatives and explore other states initiatives
- Same or increased level of biodiversity after introduction of biocontrol
- Reduced mosquito larvae counts in random sampling

E. Larval control

Current Program:
- Biorational larvicides, Bacillus thuringiensis var. israelensis (Bti), Bacillus sphaericus (Bs), and methoprene
- Surveillance of the nearly 2,000 breeding points in the County
- 15,000 inspections of breeding sites and other surveillance findings(includes catch basins and sumps)
- Approximately 4,000 acres of the County's salt marshes aerial larvicided

Long-Term Plan Recommendations:
- Increased surveillance
- Surveillance of the 2,000 breeding points in the County
- 15,000 inspections of breeding sites and other surveillance findings
- Identify problem breeding sites
- Expanded catch basin and recharge basin larviciding
- Implementation of ecological controls
- Implementation of formal resistance testing and management
- Water management- 75% percent reduction goal in acreage treated

Indicators of Success
- Number of inspections/surveillance events.
- Area larvicided (frequency and extent).
- Record and analyze dip counts in relation to reduction in treatments (results).
- Annual larvicide efficacy reports (results).
- Reduced adulticide events expected after successful larvicide control in known problem areas.

F. Adult control (only if necessary)

Current Program:

- Resmethrin, sumithrin, malathion, permethrin and natural pyrethrin
- Adulticide-directed surveillance, decision-making procedures, and efficacy and resistance testing

Long-Term Plan Recommendations:

- Criteria for spraying
  - Evidence of mosquitoes biting humans – service requests mapped
  - Verification of problem-New Jersey trap counts > 25 females /night
  - CDC light trap counts > 100; Landing rates of one to five per minute
  - Control is technically feasible Weather conditions suitable (no rain, winds<10 mph, temperature 65 ° or above)
- Improved spray technology ("Adapco Wingman") to minimize pesticide application and optimize mosquito control.
- Augment the New Jersey light trap network from 27 to 30. Expand as resources allow (see surveillance).
- Increase the number of CDC light traps from 27 to 35. Expand as resources allow (see surveillance).
- Increase CDC trap-nights to 105 per week.
- Reduce adulticide usage (currently less than 2% of County in non-emergency situations).

Indicators of Success

- Reduction in adulticide usage.
- Efficacy tests post treatment indicate 90 – 99% population reduction.
- Efficacy tests posted annually on county web page and in annual reports.
- Aerial application efficacy released within a week or so of the application.
- Post health emergency reductions in the parity and infection rates for the target species (if staff and lab resources available).

G. Water Management:

Current Program

- Hand maintenance/machine maintenance limited to < 200,000 linear ft/yr
- Machine work limited to repair and replacement of existing structures
- No new machine ditching
- Machine maintenance limited to 50,000 ft/year (no more than 50 affected acres), and only when essential for public health or ecological reasons.
- Natural Process (No action/ reversion)
- Culvert repair/ maintenance when tidally restricted
- Stop gap ditch plug

Long-Term Plan Recommendations

- Develop a strategy for managing Suffolk County's 17,000 acres of tidal wetlands, irrespective of Vector Control concern (goal: 12-year implementation window).
- Reversion priorities, allowing natural processes to fill ditches (approx. 4,000 acres; no vector control).
- Candidates for possible restoration/water management (currently routinely larvicided; approx. 4,000 acres). Marsh health is paramount objective.
- Areas requiring more assessment (approx. 9,000 acres); low-impact best management practices are possible.
- The pre-existing policy of "no new ditching" will be continued
- Less than four percent of the County's tidal wetlands (~ 600 acres) subject to machine ditch maintenance over the next decade.

Indicators of Success
Implementation of Plan recommendations (above).

4) Wetlands Stewardship Program – Accomplishments and Plans

Long-Term Plan Recommendations

- Develop a comprehensive assessment and management plan for the 17,000 acres of tidal wetlands within three years
- Ensure the protection and preservation of functions, values, and health
- Use Vector Control Wetlands Management Plan as foundation (Goodbred Report; primary study area results)
- Inventory/assess wetlands County-wide
- Review and evaluate major wetland restoration projects
- Implement early action demonstration projects
- Develop Long-term strategies

Indicators of Success

- Existence/adoption of strategy
- Acres/subsystems assessed
- Acres /subsystems restored
- Integrated plans implemented

5) Recommended Plan Updates and Amendments

Plan updates and amendments will be made, as needed. Updates may be recommended by involved agencies, the Citizens Advisory Committee, Technical Advisory Committee, and/or Wetlands Stewardship Committee. Updates require review/approval of the Steering Committee.

The Triennial Plan will be reviewed by the Steering Committee, and, as with the Annual Plan of Work, submitted to the Legislature for approval. This ensures that necessary adjustments to the Long-Term Plan are incorporated in the same open and public process that produced the Long-

Term Plan, and that adequate review is undertaken prior to adoption of any consequential change.

In addition to these formal reporting requirements, SCVC and the ABDL should release various program reports each year that describe the effectiveness of the ongoing program. Surveillance program summaries, efficacy testing results, and wetlands management as-builts are all examples of important products that will be made available. Another set of documents likely to be of public interest would be sampling and other permit and project compliance information for wetlands management projects. The best means for public release would be to post in a permanent fashion on the SCVC web site.

10.3 Examples of Areas of Adaptive Management

Clearly, as technology evolves, SCVC methodologies need to change. Upgrades of particular models, application technologies (such as nozzles and aircraft), implementation of new means of conducting WNV surveillance, and new mapping or other computer capabilities are clearly alterations that are countenanced within the Long-Term Plan. If new pesticides are developed, and it can be shown that they represent equal or less risk to human health and the environment, these products may also be incorporated under the existing Long-Term Plan. However, major changes in policy or the adoption of a treatment means not discussed within the Long-Term Plan (genetic manipulation of mosquitoes or mosquito predators, for example) would almost certainly constitute a major change in the Long-Term Plan, and require substantial review under appropriate procedures and statutes.

Wetlands in general, and fresh water wetlands in particular, are areas where adaptive management is required. At this time, regulatory interpretations limit SCVC's ability to address issues relating to mosquito breeding and presence in these areas. There are three key, interrelated issues that will be addressed over time under the adaptive management procedures of the Long-Term Plan:

- Adjustments to current SCVC procedures to account for vulnerable species or areas. SCVC, NYSDEC, the towns, and other interest groups will work closely together to identify specific organisms or areas where sensitive mosquito management is preferred.

SCVC has already worked with NYSDEC to identify tiger salamander habitats, for example, and has adjusted its activities to reduce the chances that harm might befall these creatures. Similarly, other species of special interest, or even certain sites of special concern, will be discussed with the aim of finding consensus between perceived mosquito management and environmental protection needs.

- Related to this is SCVC's interest in reconsidering the buffers set cooperatively by SCVC and NYSDEC to fulfill regulatory needs regarding wetlands and pesticides labels. SCVC believes that a more nuanced description of many of the wetland areas it undertakes its work can allow for reconsideration of the existing buffers. At this time, there is a fixed 150 foot buffer from fresh water wetlands, and 100 foot buffer from open water in general. The results of the Long-Term Plan risk assessment should be useful in determining, on a scientific basis, exactly what distance from open water will serve as protection from impacts.

- Additionally, NYSDEC may wish to consider the current prohibition of habitat manipulation in fresh water wetland settings. These regulations have served as strong tools to preserve fresh water wetlands, but it may not be in society's overall interest to continue to use pesticides as the preferred means of managing mosquitoes in fresh water environments. Alternatives to pesticide use should be considered, and, if found acceptable by the NYSDEC, implemented if they accord with the principles espoused in the Wetlands Management Plan, Best Management Practices Manual, and the impact analysis presented in the Draft Generic Environmental Impact Statement, as developed by the Long-Term Plan.

Perhaps the key wetlands issue that lends itself to adaptive management is the concept of salt marsh health, especially as it relates to diversity of habitat and organisms for Suffolk County's marshes, in particular. National understanding of these concepts has been advanced recently through on-going research, but much work in salt marshes appears to be site specific. Some research needs to be conducted in Suffolk County marshes, particularly those on the south shore, to determine if the general concepts that seem to apply to many other areas also apply to these marshes and their own peculiar, microtidal setting. Fostering a greater conceptual understanding

of the general processes that operate in County marshes can only aid to the restoration efforts that SCVC intends to either conduct or assist in. This important topic will be addressed by the Wetlands Stewardship Committee. Once completed, this work will be used to develop the comprehensive County marsh management plan, which will be the basis of the overarching Integrated Wetlands Management program. It is quite likely that some changes to the proposed wetlands management program set forth here in the Long-Term Plan will be made to address the intricacies of the Integrated Marsh Management program.

In the short-term, one element of adaptive management that will need to be incorporated into the Long-Term Plan is the development of the mosquito management program for FINS. This program is expected to be consonant with the program described here, but will have various nuances that are developed based on resource and access limitations, or on special policy considerations that are required due to the National Park presence.

11. Resource Commitments

11.1 Personnel

Because Suffolk County is embarking on a new program, with numerous technical and administrative uncertainties (i.e., leveraging surveillance resources between SCDHS & SCDPW), staffing cannot be projected with precision. However, it is clear that immediate needs exist, and long-term needs are likely to be significant as such, in the proposed 2006 County budget, the Suffolk County Executive added two positions to SCVC (an Entomologist and an Engineering Aide), which were described as the highest priority positions needed to begin implementing the Long-Term Plan. The SCDHS ABDL also received the two highest priority requested positions.

Positions identified by SCVC to fully meet needs for implementation of all elements of the Long-Term Plan would require substantially more resources. Some of the salary commitment could meet the requirements associated with Suffolk County Water Quality Protection and Restoration Program (1/4% sales tax) funding (if available). Staff associated with the on-going construction-restoration activities in wetlands may also be eligible for cost sharing under various environmental grant programs at many levels of government. Cost share initiatives with private entities (e.g., non-profit organizations) will also be pursued.

The ABDL laboratory positions, when fully needed, could also require substantially greater financial resources. The ABDL position request assumes the construction and full staffing of the requested BSL-3 facility. A major contingency is the development of an alternative surveillance tool for WNV detection to replace dead bird analysis.

11.2 Equipment and Other Capital Needs

Marsh management equipment needs appear to require approximately $250,000. These kinds of equipment often qualify for match funding through State restoration bonds and other funding opportunities.

Installation of at least one adjunct Identification Station in an existing County facility to serve the East End is warranted. Costs associated with such an installation (some room modifications,

such as paint and installation of appropriate plumbing, and equipment such as refrigerators and microscopes) appear to be relatively minor (less than $25,000).

Planning for, procuring, and constructing a BSL-3 laboratory is a major undertaking. This cost needs to be carefully estimated before the County commits to such a project.

11.3 Ancillary Funding

Associated with changes in wetlands management are many ancillary funding commitments that the County should be aware of, even if it does not intend to commit to at this time. Some of these costs may be absorbed by other Departments in the regular course of duties, and some may require new positions (if addressed by County personnel) or consultant costs (if professional services are arranged for). Although SCVC is proposing a major staff expansion to address marsh management, much of that staff will be committed to "present-day" projects. Those active construction-restoration projects are, in fact, eligible for support from Federal, State, and even County restoration and environmental management funds. Staff support for the Wetlands Stewardship Committee is also likely to be necessary. It may be that the slow down in wetlands management implementation may allow some funds to be shifted to this essential planning function.

Project planning and on-going monitoring activities (pre- and post-project) are not so eligible in most cases. These costs can be significant. Despite substantial in-kind contributions from County, USFWS, and Stony Brook University staff, OMWM monitoring at Wertheim has averaged approximately $100,000 per year in consultant costs (even with reliance on low-paid interns for routine work). These requirements will multiply, as the number of completed projects rises, where monitoring is still required, and the pace of active projects increases with experience.

Planning for these very complicated projects may involve computer modeling and intricate GIS applications. It also may involve making good and careful environmental measurements – pre-project monitoring, but prior to any regulatory requirements. The County is fortunate that other levels of government and several non-governmental organizations are expressing interest in assisting the County with project planning, and may also assist in monitoring duties.

However, it is very important for the County to discuss with New York State the need to recognize fiscal realities in project monitoring. As expertise with local OMWM is obtained, it is requested that the State will continue to work cooperatively with Suffolk County to refine (streamline) monitoring approvals.

Other on-going costs will include expenses associated with outreach efforts. Publications and meetings needed to improve communications with the public and specialized audiences such as stormwater professionals could easily amount to $100,000 to $125,000, depending on the frequency of meetings (and amenities offered at such meetings), the quality of brochures and other printed matter, and potential production costs for radio or video outreach. Creation of fish stocking efforts will also result in on-going, additional programmatic costs (estimated here to be $10,000). The resistance program for larvicides and adulticides could also be a significant, on-going cost. This was approximated here as $100,000, although the cost should be significantly less. Maintenance of the Adapco system will require some monies each year (estimated here at $5,000). Support for on-going professional education (mostly travel expenses) may be as much as $10,000 or more for both SCDPW and SCDHS over the course of a year.

Research associated with tracking bird migration, important for determining potential routes for EEE dispersal, could very well be a substantial, albeit one-time, expense. The estimate listed here is $375,000 (estimated to be supported by the County and State and Federal sources – although the County is assigned approximately two-thirds of the cost share).

Costs associated with the Long-Term Plan that were not assigned a County share include marsh restoration grant funds (State and Federal sources) (expected to exceed $1 million), support for County marsh health programmatic policy development (State and Federal sources) (estimated at $250,000), and development of Unit Management Plans for State marshes (estimated cost of $1 million). In addition, better tire management by towns and other waste management authorities could increase costs substantially (removing an additional 5,000 tires from the County's woods might cost as much as $25,000 per year for disposal).

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12. Implementation Recommendations

In order for the Long Term Plan to be successfully implemented, a number of actions are required to be taken by involved stakeholders, including SCDHS and SCDPW, local elected officials from the County, townships and villages, State and Federal agencies, involved non-governmental stakeholders such as Ducks Unlimited and The Nature Conservancy, and the general public. These recommendations are summarized in Table 24 following this section of the Long-Term Plan.

12.1 Actions by County Program Managers in SCDPW and SCDHS

Public Outreach

1. Increase public contact and vector control information dissemination through public meetings, brochures, fact sheets, etc., and actively participate in Mosquito Awareness Week.

2. Conduct outreach programs and information dissemination in Spanish and other languages in areas of the county where English may not be the residents' first language.

3. Expand public and municipal education outreach by incorporating tire management as a means of vector control. Include tire collection as part of routine sanitation or maintenance activities.

4. Partner with Cornell Cooperative Extension Service in educational mailings/meetings targeted to specific audiences for implementation of irrigation practices that prevent or minimize the potential for mosquito breeding.

5. Conduct tailored outreach programs to municipal highway departments and programs regarding stormwater structures as mosquito habitat (including SCDPW).

6. Increase efforts to educate parties responsible for stormwater management to utilize methods that do not increase the potential for mosquito breeding or introduce contaminants to wetlands.

7. Create Listserv for adulticide applications in order to expand public notification procedures.

8. Provide the resources necessary to update and improve the SCVC website in order to provide the public with timely information and descriptions of vector control activities being conducted in various areas of the county.

9. Be aware of opportunities to create useful PSA presentations associated with the vector control program.

10. Expand outreach efforts to areas that receive more frequent adulticide applications per Section 3.

Surveillance

11. Provide the necessary resources and support implementation of efforts for increased frequency and greater number of recharge basin sampled.

12. Provide the necessary resources and support implementation of efforts for increasing the number of catch basin sampled.

13. SCVC and the ABDL should implement expanded CDC light trap surveillance as outlined in Section 3.

14. Establish New Jersey trap stations on Fire Island as outlined in Section 3.

15. Conduct the necessary research and fieldwork to locate ambient trap stations (New Jersey traps preferred).

16. Provide the necessary support and resources for SCVC to establish Identification Stations in existing County facilities.

17. Provide the necessary support and resources for SCVC and the ABDL to develop and conduct a Fishers Island disease surveillance program.

18. Encourage and support efforts by the QA/QC team and the ABDL to develop an alternative WNV technique to replace dead bird analysis.

19. Encourage and support SCVC efforts to establish landing rate sites.

20. SCVC program managers should reconsider whether the benefits associated with the VCMS system exceed the difficulties in translating its data in GIS.

21. The ABDL should produce annual reports on surveillance analysis.

Biocontrol

22. SCDPW should provide sufficient resources for SCVC to replace use of Gambusia with fathead minnows, including development of the capability of farming the fish in-house if economically viable.

23. SCVC should follow developments in New Jersey, where culturing of predatory copepods is being attempted, and implement this technique if feasible.

Water Management

24. SCVC should implement the BMP Manual.

25. SCVC's should plan for and conduct projects to displace aerial larviciding.

26. The long-term goal must be to assess 9,000 acres of marsh for needed management activities.

27. SCVC and the SCDHS Office of Ecology should work with NYSDEC to evaluate fresh water management prohibitions.

28. SCVC should work with town natural resource officials and other stakeholders to implement appropriate water management projects.

29. County program managers should continue to work with the Wetlands Stewardship Committee to refine a definition of County marsh health.

30. County program managers should continue to work with the Wetlands Stewardship Committee to create a comprehensive marsh management plan, to be the basis of an Integrated marsh management program.

31. Reports on marsh management projects should be made available through the Internet.

Larviciding

32. SCVC should coordinate cooperative actions regarding vulnerable species in fresh water habitats.

33. SCVC should record and analyze dip counts to relate to treatment reductions.

34. If New Jersey research supports the effort, SCVC should conduct its own copepod research (with a catch basin focus) to reduce larvicide applications.

35. Develop a means of conducting routine efficacy testing on individual applications.

36. Prepare and issue an appropriate means for enabling a contract with an organization capable of developing a professional resistance detection program.

37. On an annual basis, SCVC should prepare larvicide efficacy reports.

Adulticiding

38. SCVC should maintain and upgrade the recently installed Adapco Wingman system.

39. The County should implement the proposed efficacy program (Section 7).

40. SCVC should establish the comprehensive efficacy program outlined in Section 7.

41. The County should support bird dispersal and migration research as a means of understanding EEE dynamics.

42. SCVC should conduct necessary research and outreach to establish an effective means for Culex adult mosquito control.

43. SCVC should install/purchase GPS for all equipment and personnel.

44. SCVC, after careful analysis of the risk assessment results, should work with NYSDEC regarding wetlands setback requirements based on natural resources considerations.

45. Prepare and issue an appropriate means for enabling a contract with an organization capable of developing a professional resistance detection program.

Administration

46. SCDHS and SCDPW administrators should redistribute surveillance responsibilities.

47. SCVC should implement its organizational restructuring.

48. SCDPW and SCDHS should provide budgetary support to enable SCVC and the ABDL to establish needed new positions.

49. SCDHS should plan the ABDL BSL-3 lab and construct, if feasible.

50. SCDPW and SCDHS should encourage personnel to participate in extensive continuing education programs and scientific meetings.

51. SCVC and SCDHS must produce required reports (such as the Triennial Report, efficacy, water management, and surveillance results, and the Annual Plan of Work) and public outreach material (such as updated brochures and website information sets).

12.2 Actions by County Executive and Legislature

Public Outreach

1. Provide the resources to allow for implementation of irrigation practices education (through Cornell Cooperative Extension).

2. Establish responsibility in a particular agency to implement private storm water system maintenance education.

3. Provide public relations opportunities to enhance the public image of SCVC to encourage public and government cooperation with its activities.

Surveillance

4. Provide necessary funds and institutional approvals to establish Identification Stations within existing County properties.

5. Reconsider County vehicle policies regarding overnight, at-home possession, to allow for more efficient surveillance activities.

6. Provide sufficient number and field appropriate vehicles for surveillance activities.

Water Management

7. Actively support the use of Water Quality Protection (1/4% sales tax) Funds for wetlands initiatives.

8. Take the necessary steps to establish, support, and participate in the Wetlands Stewardship Committee.

9. The County should consider policy changes that could result in programmatic means that will improve County marsh health, such as permitting of coastal septic systems, or other options that may indirectly affect coastal water quality of marsh environments.

Adulticiding

10. The county should provide necessary funds to support bird dispersal and migration research.

Administration

11. The county should provide institutional support to allow the redistribution of surveillance responsibilities.

12. Implement departmental reorganizations.

13. Fund and establish essential new positions.

14. Provide the means to plan and construct ABDL BSL-3 lab.

15. Reconsider current policies regarding employee travel to allow for appropriate professional development.

12.3 Actions by Other Local Government

Source Control

1. Encourage towns and villages to work with SCVC to address storm water structures and their potential to breed mosquitoes.

2. Improve the waste management and parks departments tire management practices.

Water Management

3. Towns and other levels of government should participate in the Wetlands Management Plan to work with SCVC and others to implement appropriate water management projects.

4. Towns and other interested levels of government should participate in and support the Wetlands Stewardship Committee.

5. Town natural resource divisions should participate in the Wetlands Subcommittee and provide technical support to and critical review of proposed water management projects.

6. Towns and other interested levels of government should seek to develop programmatic means that will improve County marsh health, such as zoning and other planning steps that can affect coastal water quality and marsh environments.

Larviciding

7. Town natural resource agencies should conduct cooperative actions regarding vulnerable species in fresh water habitats, such as sharing any information relating to sensitive species or habitats.

Adulticiding

8. Town natural resource agencies should provide material and political support for bird dispersal and migration research.

12.4 Actions by State or Federal Governments

Water Management

1. NYSDEC: NYSDEC should work with SCVC to evaluate fresh water marsh management options in light of other states' regulations and experiences, as well as particular habitat and ecological settings in Suffolk County.

2. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies should work with SCVC, towns, and other organizations to identify, permit, and implement appropriate water management projects, providing technical input and guidance where expertise is available.

3. NYSDEC: NYSDEC is encouraged to formally participate in Wetlands Stewardship Committee.

4. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies should continue to participate in the Wetlands Subcommittee.

5. NYSDEC and others (NYSDOS, NYSERDA, USEPA, USACOE): These agencies should actively support the use of restoration grant funds (and other available funding mechanisms) for potential wetlands projects.

6. NYSDEC: Means to issue general permits or other means of expedited project review, where appropriate, should be explored with SCVC and other interested parties, to implement low-impact BMPs.

7. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies need to support research and other mechanisms (workshops, demonstration projects,

planning grants) that will result in the development of programmatic means to improve County marsh health.

8. NYSDEC: A priority to enable implementation of progressive water management in the County is that NYSDEC develops Unit Management Plans for State marsh holdings.

Larviciding

9. NYSDEC and others (USEPA, USFWS, NPS): Natural resource specialists should work with SCVC and other local experts to identify potentially vulnerable species in fresh water habitats.

10. NYSDEC: NYSDEC is encouraged to review the existing literature on larvicide non-target impacts, with the intent of reaching agreement with the County regarding best uses of these products.

Adulticiding

11. NYSDEC and others (USEPA, USFWS, NPS): provide material and political support for Long Island-relevant bird dispersal and migration research.

12. NYSDEC: NYSDEC and SCVC should carefully examine the results of the risk assessment to determine if current wetlands and aquatic habitat setbacks should be reconsidered.

12.5 Actions by Other Interested Parties (NGOs, Civic Associations, interested individuals)

Water Management

1. Interested parties should work with SCVC and towns to implement appropriate water management projects.

2. Interested parties should participate in the Wetlands Subcommittee.

3. Interested parties should work with the County and towns to identify implementable programmatic guidelines that can be demonstrated to lead to improvements in County marsh health. Sites are needed for demonstration projects, and appropriate background research/studies are needed.

4. Interested parties should seek to work with the County in developing a comprehensive County marsh management plan, to serve as the basis for the Integrated marsh Management program.

5. Interested parties should seek to serve on the Wetlands Stewardship Committee.

Larviciding

6. Interested parties with appropriate natural resource expertise should assist SCVC in identifying potentially vulnerable species in fresh water habitats.

Adulticiding

7. Interested parties should support (and potentially assist in) bird dispersal and migration research.

Table 24. Action Items Summary

Section Headings	Agencies	Action	Timetable	Cost Estimate
Public Outreach	SCDPW	1. Increase public contact and vector control information dissemination through public meetings, brochures, fact sheets, etc., and actively participate in Mosquito Awareness Week.	2007+	~$10K/yr
		2. Partner with Cornell Cooperative Extension Service in educational mailings/meetings targeted to specific audiences for implementation of irrigation practices that prevent or minimize the potential for mosquito breeding.	2007+	~$5K/yr
		3. Expand public and municipal education outreach by incorporating tire management as a means of vector control. Include tire collection as part of routine sanitation or maintenance activities.	2007+	~$5K/yr
		4. Conduct tailored outreach programs to municipal highway departments and programs regarding stormwater structures as mosquito habitat (including SCDPW).	2007+	~$5K/yr
		5. Increase efforts to educate parties responsible for storm water management to utilize methods that do not increase the potential for mosquito breeding or introduce contaminants to wetlands.	2007+	~$5K/yr
		6. Create Listserv for adulticide applications in order to expand public notification procedures.	2007+	
	SCDHS	1. Increase public contact and vector control information dissemination through public meetings, brochures, fact sheets, etc., and actively participate in Mosquito Awareness Week.	2007+	~$10K/yr
		2. Conduct outreach programs and information dissemination in Spanish and other languages in areas of the county where English may not be the residents' first language.	2007+	~$50K 1st yr, ~$25K/yr
		3. Expand public and municipal education outreach by incorporating tire management as a means of vector control. Include tire collection as part of routine sanitation or maintenance activities.	2007+	~$5K/yr
		4. Partner with Cornell Cooperative Extension Service in educational mailings/meetings targeted to specific audiences for implementation of irrigation practices that prevent or minimize the potential for mosquito breeding.	2007+	~$5K/yr
		5. Conduct tailored outreach programs to municipal highway departments and programs regarding stormwater structures as mosquito habitat (including SCDPW).	2007+	~$5K/yr
		6. Increase efforts to educate parties responsible for storm water management to utilize methods that do not increase the potential for mosquito breeding or introduce contaminants to wetlands.	2007+	~$5K/yr
		7. Be aware of opportunities to create Useful PSAs associated with vector control.	2007+	
		8. Expand outreach efforts to areas that receive more frequent adulticiding.	2007+	~$5K/yr.
	SCDEE	1. Increase public contact and vector control information dissemination through public meetings, brochures, fact sheets, etc., and actively participate in Mosquito Awareness Week.	2007+	~$5K/yr
		2. Expand public and municipal education outreach by incorporating tire management as a means of vector control. Include tire collection as part of routine sanitation or maintenance activities.	2007+	~$5K/yr
		3. Conduct tailored outreach programs to municipal highway departments and programs regarding stormwater structures as mosquito habitat (including SCDPW).	2007+	~$5K/yr
		4. Increase efforts to educate parties responsible for storm water management to utilize methods that do not increase the potential for mosquito breeding or introduce contaminants to wetlands.	2007+	~$5K/yr

Section Headings	Agencies	Action	Timetable	Cost Estimate
	County Executive and Legislature	1. Provide the resources necessary to update and improve the SCVC website in order to provide the public with timely information and descriptions of vector control activities being conducted in various areas of the county. 2. Provide the resources to allow for implementation of irrigation practices education (through Cornell Co-operative Extension). 3. Establish responsibility in a particular agency to implement private storm water system maintenance education. 4. Provide public relations opportunities to enhance the public image of SCVC to encourage public and government cooperation with its activities.	2007 2007 2007 2007+	
Surveillance	SCDPW	1. Establish New Jersey trap stations on Fire Island as outlined in Section 3. 2. Conduct the necessary research and fieldwork to locate ambient trap stations (New Jersey traps preferred). 3. Encourage and support SCVC efforts to establish landing rate sites. 4. SCVC program managers should reconsider whether the benefits associated with the VCMS system exceed the difficulties in translating its data in GIS. 5. SCVC and the ABDL should implement expanded CDC light trap surveillance as outlined in Section 3. 6. Encourage and support efforts by the QA/QC team and the ABDL to develop an alternative WNV technique to replace dead bird analysis.	2007 2008 2007 2007 2007 2007-2008	
	SCDHS	1. Establish New Jersey trap stations on Fire Island as outlined in Section 3. 2. Conduct the necessary research and fieldwork to locate ambient trap stations (New Jersey traps preferred). 3. The ABDL should produce annual reports on surveillance analysis. 4. SCVC and the ABDL should implement expanded CDC light trap surveillance as outlined in Section 3. 5. Encourage and support efforts by the QA/QC team and the ABDL to develop an alternative WNV technique to replace dead bird analysis.	2007 2007 2007+ 2007 2007-2008	
	County Executive and Legislature	1. Provide the necessary resources and support implementation of efforts for increased frequency and greater number of recharge basin sampled. 2. Provide the necessary resources and support implementation of efforts for increasing the number of catch basin sampled. 3. Provide the necessary support and resources for SCVC to establish Identification Stations in existing County facilities. 4. Provide the necessary support and resources for SCVC and the ABDL to develop and conduct a Fishers Island disease surveillance program. 5. Re-consider County vehicle policies regarding overnight, at-home possession, to allow for more efficient surveillance activities. 6. Provide sufficient number and field appropriate vehicles for surveillance activities.	2007 2007 2007-2008 2007-2008 2007 2007	
Source Control	Other Local Government	1. Encourage towns and villages to work with SCVC to address stormwater structures and their potential to breed mosquitoes. 2. Improve the waste management and parks departments tire management practices.	2007+ 2007+	~$25K/yr Countywide
Water Management	SCDPW	1. SCVC should implement the BMP Manual. 2. SCVC should plan for and conduct projects to displace aerial larviciding. 3. The long-term goal must be to assess 9,000 acres of marsh for needed management activities 4. SCVC and the SCDHS Office of Ecology should work with NYSDEC to evaluate fresh water management prohibitions. 5. SCVC should work with towns and other stakeholders to implement appropriate water management projects. 6. County program managers and the Wetlands Stewardship Committee should develop programmatic means of improving County marsh health. 7. County program managers and the Wetlands Stewardship Committee should develop a comprehensive marsh management plan.	2007+ 2007+ 2007+ 2007-2008 2007+ 2007-2009 2007-2009	 ~$100K ~$200K

Section Headings	Agencies	Action	Timetable	Cost Estimate
	SCDHS	1. The long-term goal must be to assess 9,000 acres of marsh for needed management activities over 10 years. 2. SCVC and the SCDHS Office of Ecology should work with NYSDEC to evaluate fresh water management prohibitions. 3. County program managers and the Wetlands Stewardship Committee should develop programmatic means of improving County marsh health. 4. County program managers and the Wetlands Stewardship Committee should develop a comprehensive marsh management plan.	2007+ 2007-2009 2007-2009 2007-2009	
	SCDEE	1. The long-term goal must be to assess 9,000 acres of marsh for needed management activities over 10 years. 2. County program managers and the Wetlands Stewardship Committee should develop programmatic means of improving County marsh health. 3. County program managers and the Wetlands Stewardship Committee should develop a comprehensive marsh management plan.	2007+ 2007-2009 2007-2009	
	County Executive and Legislature	1. Actively support the use of Water Quality Protection (1/4% sales tax) Funds for wetlands initiatives. 2. Take the necessary steps to establish, support, and participate in the Wetlands Stewardship Committee. 3. The County should consider policy changes that could result in programmatic means that will improve County marsh health, such as permitting of coastal septic systems, or other options that may indirectly affect coastal water quality of marsh environments.	2007+ 2007 2007-2009	
	Other Local Government	1. Towns and other levels of government should participate in the Wetlands Management Plan to work with SCVC and others to implement appropriate water management projects. 2. Towns and other interested levels of government should participate in and support the Wetlands Stewardship Committee. 3. Town natural resource divisions should participate in the Wetlands Subcommittee. 4. Towns and other interested levels of government should seek to develop programmatic means that will improve County marsh health, such as zoning and other planning steps that can affect coastal water quality and marsh environments.	2007+ 2007+ 2007+ 2007-2009	
	State or Federal Governments	1. NYSDEC: NYSDEC should work with SCVC to evaluate fresh water marsh management prohibitions in light of other states' regulations and experiences, as well as particular habitat and ecological settings in Suffolk County. 2. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies should work with SCVC, towns, and other organizations to identify, permit, and implement appropriate water management projects, providing technical input and guidance where expertise is available. 3. NYSDEC: NYSDEC should make a policy decision to participate in Wetlands Stewardship Committee. 4. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies should continue to participate in the Wetlands Subcommittee. 5. NYSDEC and others (NYSDOS, NYSERDA, USEPA, USACOE): These agencies should actively support the use of restoration grant funds (and other available funding mechanisms) for potential wetlands projects. 6. NYSDEC: Means to issue general permits or otherwise streamline project review process, where appropriate, to implement low-impact BMPs on an expedited basis need to be explored with SCVC and other interested parties. 7. NYSDEC and others (NYSDOS, USEPA, USACOE, USFWS, NPS): These agencies need to support research and other mechanisms (workshops, demonstration projects, planning grants) that will result in the development of programmatic means to improve County marsh health. 8. NYSDEC: A priority to enable implementation of progressive water management in the County is that NYSDEC develops Unit Management Plans for State marsh holdings.	2007-2008 2007+ 2007 2007+ 2007+ 2007+ 2007-2009 2007+	 >$1million ~$250K ~$1 million

Section Headings	Agencies	Action	Timetable	Cost Estimate
	Other Interested Parties (NGOs, Civic Associations, interested individuals)	1. Interested parties should work with SCVC and towns to implement appropriate water management projects. 2. Interested parties should participate in the Wetlands Subcommittee. 3. Interested parties should seek to participate on the Wetlands Stewardship Committee 4. Interested parties should work with the County and towns to identify implementable programmatic guidelines that can be demonstrated to lead to improvements in County marsh health. Sites are needed for demonstration projects, and appropriate background research/studies are needed. 5. Interested parties should assist in the development of a comprehensive marsh management plan.	2007+ 2007+ 2007+ 2007-2009 2007-2009	
Biocontrol	SCDPW	1. SCDPW should provide sufficient resources for SCVC to replace use of Gambusia with fathead minnows, including development of the capability of farming the fish in-house if economically viable. 2. SCVC should follow developments in New Jersey, where culturing of predatory copepods is being attempted, and implement this technique if feasible.	2007-2008 2007-2009	~$10K/yr

Section Headings	Agencies	Action	Timetable	Cost Estimate
Larviciding	SCDPW	1. SCVC should coordinate cooperative actions regarding vulnerable species in fresh water habitats. 2. SCVC should record and analyze dip counts to relate to treatment reductions. 3. If New Jersey research supports the effort, SCVC should conduct its own copepod research (with a catch basin focus) to reduce larvicide applications. 4. Develop a means of conducting routine efficacy testing on individual applications. 5. Prepare and issue an appropriate means for enabling a contract with an organization capable of developing a professional resistance detection program. 6. On an annual basis, SCVC should prepare larvicide efficacy reports.	2007+ 2007+ 2007-2009 2007+ 2007 2007+	~$50K??/yr
	SCDHS	Participate in cooperative actions regarding vulnerable species in fresh water habitats.	2007+	
	SCDEE	Participate in cooperative actions regarding vulnerable species in fresh water habitats.	2007+	
	Other Local Government	Town natural resource agencies should conduct cooperative actions regarding vulnerable species in fresh water habitats, such as sharing any information relating to sensitive species or habitats.	2007+	
	State or Federal Governments	1. NYSDEC and others (USEPA, USFWS, NPS): Natural resource specialists should work with SCVC and other local experts to identify potentially vulnerable species in fresh water habitats. 2. NYSDEC is encouraged to review the existing literature on larvicide non-target impacts, with the intent of reaching agreement with the County regarding best uses of these products	2007+ 2007	
	Other Interested Parties (NGOs, Civic Associations, interested individuals)	Interested parties with appropriate natural resource expertise should assist SCVC in identifying potentially vulnerable species in fresh water habitats.	2007+	

Adulticiding	SCDPW	1. SCVC should maintain and upgrade the recently installed Adapco Wingman system. 2. SCVC should establish the comprehensive efficacy program outlined in Section 8. 3. The County should support bird dispersal and migration research as a means of understanding EEE dynamics. 4. SCVC should conduct necessary research and outreach to establish an effective means for Culex adult mosquito control. 5. SCVC should install/purchase GPS for all equipment and personnel. 6. SCVC, after careful analysis of the risk assessment results, should work with NYSDEC regarding wetlands setback requirements based on natural resources considerations. 7. Prepare and issue an appropriate means for enabling a contract with an organization capable of developing a professional resistance detection program.	2007+ 2007 2007-2009 2007-2008 2007-2008 2007-2008 2007	~$5K/yr ~$125K ~$250K ~$50K??/yr
	SCDHS	1. The County should support bird dispersal and migration research as a means of understanding EEE dynamics.	2007-2009	~$125K
	County Executive and Legislature	The County should provide necessary funds to support bird dispersal and migration research.	2007	
	Other Local Government	Town natural resource agencies should provide material and political support for bird dispersal and migration research.	2007-2009	
	State or Federal Governments	1. NYSDEC and others (USEPA, USFWS, NPS): provide material and political support for Long Island-relevant bird dispersal and migration research. 2. NYSDEC: NYSDEC and SCVC should carefully examine the results of the risk assessment to determine if current wetlands and aquatic habitat setbacks should be reconsidered.	2007-2009 2007-2008	$125K
	Other Interested Parties (NGOs, Civic Associations, interested individuals)	Interested parties should support (and potentially assist in) bird dispersal and migration research.	2007-2009	
Administration	SCDPW	1. SCDHS and SCDPW administrators should redistribute surveillance responsibilities. 2. SCVC should implement its organizational .restructuring. 3. SCDPW should provide budgetary support to enable SCVC to establish needed new positions. 4. SCDPW should encourage personnel to participate in extensive continuing education programs and scientific meetings. 5. SCVC and SCDHS must produce required reports (such as the Triennial Report, efficacy results, etc., and the Annual Plan of work) and public outreach material (such as updated brochures and website information sets).	2007 2007 2007-2011 2007+ 2007+	 ~$600K/yr ~$25K/yr ~$25/K/yr
	SCDHS	1. SCDHS and SCDPW administrators should redistribute surveillance responsibilities. 2. SCDHS should provide budgetary support to enable the ABDL to establish needed new positions. 3. SCDHS should plan and construct the ABDL BSL-3 lab. 4. SCDHS should encourage personnel to participate in extensive continuing education programs and scientific meetings. 5. SCVC and SCDHS must produce required reports (such as the Triennial Report, efficacy results, etc., and the Annual Plan of work) and public outreach material (such as updated brochures and website information sets)	2007 2007-2011 2007-2011 2007+ 2007+	 ~$550K/yr Unk. but substantial ~$25K/yr ~$25K/yr
	County Executive and Legislature	1. The county should provide institutional support to allow the redistribution of surveillance responsibilities. 2. Implement organizational reorganization. 3. Fund and establish essential new positions. 4. Provide the means to plan and construct ABDL BSL-3 lab. 5. Reconsider current policies regarding employee out-of-state travel to allow for appropriate professional development.	2007+ 2007 2007-2011 2007-2011 2007	

Appendix 1 - Pesticide Labeling Requirements

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Section 1 – Introduction

FIFRA provides for federal control of the distribution, sale and use of pesticides. All label language must be approved by USEPA prior to a pesticide being sold or distributed in the United States. The pesticide label is the primary document for conveying general and technical information from regulatory agencies and pesticide manufacturers to mosquito control agencies, the agricultural community, the commercial service industry, and the general public. It is the one source where scientific review, regulatory oversight, and public policy are interwoven to achieve a common objective: to clearly and precisely convey information on handling, storing, applying, and disposing of pesticides in a manner conducive to good health and environmental stewardship (Whitford et al., 2001).

Pesticides are developed by the manufacturer, registered with USEPA, and sold to the public with the assumption that users read, understand, and follow instructions found on the product label. Specific information on use, personal protective equipment, environmental precautions, and storage and disposal are found on the pesticide label. The purpose of the label is to provide clear directions to allow maximum product benefit while minimizing risks to human health and the environment. All research, testing, and regulatory processes ultimately are reflected through the language on the label (NYSDEC, 2003a).

Every pesticide label includes the statement, "It is a violation of federal law to use this product in a manner inconsistent with its labeling." This language obliges the purchaser or user of any pesticide to assume all legal responsibilities for the use of the product. Further, courts of law and regulators recognize the pesticide label is a binding contract that requires the person using the product to do as exactly as directed. Terms such as must, shall, do not, and shall not mean that the user is responsible for specific actions when applying or handling the given product. Any departure from such directions is, in the eyes of the law, an illegal use of the pesticide (NYC DEIS, 2001).

"Use" means more than just the application of the pesticide. Federal and state regulations define pesticide use to include handling, mixing, loading, storage, transportation, and disposal, as well as human and environmental exposure. This all-encompassing definition covers every activity that involves a pesticide—from purchase to container disposal. Many statements on the label

result from rigorous scientific investigation and governmental regulatory decisions. Pesticide users should read, understand, and follow pesticide label directions to ensure effective pest control, personal safety, environmental protection and legal compliance (Whitford et al., 2001).

Every pesticide product must bear a label that contains the information specified in FIFRA and the regulations in 40 CFR 156.10. The contents of the label must clearly and prominently show the following (information presented here through Section 4.4 is taken from the federal regulations):

- Name, brand, and trademark under which the product is sold
- Name and address of the producer, registrant, or person for whom the product was produced
- Product Registration Number
- Producing Establishment Number – referring to the final establishment at which the product was produced or finished
- Net Contents, as set forth below:
    - The net weight or measure of content shall be exclusive of wrappers or other materials and shall be the average content unless explicitly stated as a minimum quantity.
    - If the pesticide is a liquid, the net content statement shall be in terms of liquid measure at 68 degrees Fahrenheit (°F) (20 degrees Celsius [°C]) and shall be expressed in conventional American units such as fluid ounces, pints, quarts, or gallons.
    - If the pesticide is a solid or semisolid, viscous or pressurized, or is a mixture of liquid and solid, the net content statement shall be in terms or weight expressed as pounds and ounces.

- In all cases, net content shall be stated in terms of the largest suitable units, i.e. "1 pound 10 ounces" rather than "26 ounces."

- In addition to the required units specified, the net content may be expressed in metric units.

- Variation above minimum content or around an average is permissible only to the extent that it represents deviation unavoidable in good manufacturing practice. Variation below a stated minimum is not permitted. In no case shall the average content of the packages in a shipment fall below the stated average content.

- Warning or precautionary statements. Every pesticide product label must bear on the front panel the statement "Keep Out Of Reach Of Children." However, human hazard signals and precautionary statements will vary according to the product's toxicity to humans, as discussed under "Toxicity Categories."

- Ingredient Statement, which must contain the name and percentage by weight of each active ingredient, the total percentage by weight of all inert ingredients, and , if the pesticide contains arsenic in any form, a statement of the percentages of total and water-soluble arsenic calculated as elemental arsenic. Accepted common names are to be used followed by chemical name unless the common name is widely known. In cases where the pesticide formulation changes considerably over time (degradation), the following statement must be written on the label: "Not for sale or use after [date]." The product must meet all requirements on the label through that date. Inert ingredients may need to be listed if they pose a hazard to public health or the environment.

- Use Classification, indicating whether the product is for general use, restricted use, or both. If it is a restricted use product, specific directions must follow. Other information may be required if its use is restricted to certain applicators.

- Directions for use, which must be easily read and understandable by the average person who will use them. They may appear anywhere on the label providing they may be easily read. Directions may be omitted if:

- The product is only to be used in manufacturing.
- It will not come into the hands of the public
- It has data sheets specifying products involved
- It is determined that directions are not necessary to prevent unreasonable adverse effects on humans and the environment
- It is only to be used by a physician
- It is a drug regulated under the Federal Food, Drug, and Cosmetic Act (FFDCA)
- It will only be used by formulators of pesticide

Section 2 Safety Information

Child hazard warning. The front panel of every pesticide product label must bear the statement, "Keep Out Of Reach Of Children." USEPA may waive this requirement only in cases where the likelihood of contact with children is extremely remote, or when the product is approved for use on children.

A **signal word** must appear prominently on the front of the pesticide container, providing, in essence, a one-word summary of the product's potential toxicity to humans. The three signal words, in decreasing order of toxicity, are DANGER (highly toxic), WARNING (moderately toxic), and CAUTION (slightly toxic).

A signal word is assigned on the basis of laboratory tests conducted with that particular product. Data are compiled from animal studies on exposure through ingestion, inhalation, and dermal (skin and eye) absorption. The route of exposure which shows the highest human toxicity potential determines the signal word assigned to the label. For example, if laboratory test results indicate product XYZ to be moderately toxic if ingested, highly toxic if inhaled, and slightly toxic if absorbed through the skin or eyes, the signal word would be danger based on inhalation studies, and would be DANGER.

Hazards to humans and domestic animals. Precautionary statements indicating specific hazards, routes of exposure, and precautions to be taken to avoid human and animal injury are required on the label. For example: "Harmful if swallowed, inhaled, or absorbed through the skin." Precautionary warnings might include the language, "Do not breathe vapors or spray mist;" "Avoid contact with eyes, skin or clothing;" or "Handle concentrate in a ventilated area."

The **protective clothing and equipment statement** directs the applicator to reduce the potential for exposure by using protective clothing or equipment. Most pesticide labels contain very specific instructions concerning the type of clothing that must be worn during the handling and mixing processes.

Potential routes of exposure determine the types of protective clothing designated on the label. Generally, a long-sleeved shirt, long pants, and waterproof footwear are the minimum requirements. The label will state whether specific items such as respirators and chemical-

resistant gloves, aprons, goggles, and boots are needed. Common label language includes "Wear full face shield, rubber gloves, apron, and waterproof footwear when pouring concentrate or when exposure to concentrate is possible," and "Eye protection and chemically resistant gloves and footwear, a long-sleeved shirt, and long-legged pants or coveralls are recommended."

The **Statement of practical treatment** (first aid) provides valuable information to persons at the scene of a pesticide poisoning. Some examples: "In case of contact with skin, wash immediately with plenty of soap and water;" "If swallowed, call a physician or poison control center immediately;" "Immediately wash eyes with water for at least 15 minutes and get medical attention;" "After first aid is given, take victim to clinic or hospital;" or, "If inhaled, remove victim to fresh air."

The statement of practical treatment informs physicians and emergency responders of appropriate medical procedures for poisoning victims. For example, the statement might indicate to a physician: "There is no specific antidote;" "If the product is ingested, induce emesis or stomach lavage;" or "The use of an aqueous slurry of activated charcoal may be considered." Products labeled DANGER also bear a toll-free telephone number that physicians may use for further treatment advice. Emergency telephone numbers are provided on the Material Safety Data Sheet (MSDS). The pesticide distributor or manufacturer should be contacted for the MSDS.

Section 3 Environmental Information

Environmental hazard statements are required to state the nature of potential hazards and appropriate precautions to avoid accident, injury, or damage if the product presents risks to non-target organisms or the environment. Potential hazards are determined by a series of tests that evaluate a pesticide's toxicity to wildlife such as mammals, fish, birds, aquatic invertebrates, and pollinating insects. Statements might include label language such as, "This product is highly toxic to bees," or "This product is highly toxic to fish," or "…toxic to aquatic invertebrates." To reduce the risks, the label may direct measures such as, "Do not allow drift to contact nontarget plants," or "Do not apply directly to water or wetlands."

If the pesticide has the potential to harm an endangered or threatened species or its habitat, statements will indicate where not to apply the pesticide or refer the user to an endangered species bulletin for further information. For example, the label might read "Use of this product in a manner inconsistent with the Pesticide Use Bulletin for Protection of Endangered Species is a violation of federal law," "Restrictions for the protection of endangered species apply to this product," or "If restrictions apply to the area in which this product is to be used, you must obtain the Pesticide Use Bulletin for Protection of Endangered Species for that county."

Statements on environmental impact may indicate that the product "…may travel through soil and can enter ground water," or "…has been found in ground water." The label instructions will tell how to reduce the impact on the environment: "This product may not be mixed, loaded, or used within 50 feet of all wells, including abandoned wells, drainage wells, and sink holes," or "This product has been shown to leach under certain conditions. Do not apply to sand and loamy sand soils where the water table (ground water) is close to the surface."

Section 4 Product Information

The **brand (trade) name** under which a pesticide product is sold always appears on the front panel and often is the most conspicuous part of the label.

The **name and address of the producer, registrant, or person for whom the product was produced** must be shown on the label. If the registrant's name appears on the label and the registrant is not the producer, it must be qualified by appropriate wording such as "Packed for…" "Distributed by…" or "Sold by…."

The **net weight or volume of the contents** of the formulated pesticide product is displayed prominently on the label or stamped on the container.

The product registration number appears on the label, preceded by the phrase "EPA Registration No." or "EPA Reg. No." The registration number identifies a specific pesticide product and signifies that federal registration requirements have been met. At a minimum, registration numbers consist of two sets of digits: e.g., 491-005. The first set of digits identifies the registrant. The second set represents the specific registration issued to the company by USEPA. Together, these numbers clearly identify the product.

The **establishment number** is preceded by the phrase "EPA Est." USEPA requires pesticide production sites to be registered with USEPA. A pesticide-producing establishment is assigned a USEPA establishment number that clearly identifies that location. All pesticides produced at that location must bear its USEPA establishment number on the label or container. Farm service centers that repackage bulk pesticides must be registered as pesticide-producing establishments and, as with all pesticide producers, must keep records of their pesticide production and file annual production reports.

The **ingredient statement** normally is found on the front panel of the label. It identifies the name and percentage of a pesticide product that affects the target pest. Chemical names often are complex; for example, *2-chloro-4-ethylamino-6-isopropylamino-s-triazine* is the active ingredient in the product *Atrex*. To aid communication, USEPA-approved common names may be substituted for chemical names.

Inert ingredients allow active ingredients to be formulated into many different products. As part of the formulation, they determine a product's handling properties and influence toxicity, release rates, residual activity, persistence, and methods of application. Also, there are no pest controlling claims for inert ingredients and, because product formulations are confidential, the total percent by weight of inert ingredients usually is the only information about inert ingredients found on the label.

The **formulation** of the product often appears on the front panel of the label, either near the brand name or in the general information section. Pesticides may be formulated into many products; currently, in the US, some 450 active ingredients are formulated into 25,000 different products. Information about the type of product formulation—granular, liquid flowable, dry flowable, microencapsulated, emulsifiable concentrate, etc—provides insight about application equipment, handling properties, and performance characteristics.

General-use versus restricted-use classification. USEPA may classify a certain pesticide product for restricted use due to the complexity of the designated use, concerns about environmental safety, or potential human toxicities. A restricted-use product may be bought and used only by a certified applicator or persons under the direct supervision of a certified applicator. A restricted-use statement appears conspicuously at the top of the front panel of the label to make this classification obvious. All restricted-use pesticides are identified by the following language: "For retail sale to and use only by certified applicators or persons under their direct supervision, and only for those uses covered by the certified applicator's certification."

Pesticides that remain unclassified are referred to as general-use pesticides and may be purchased by the public. Most pesticides used by homeowners are general-use products. However, there is no positive statement on labels approving the chemical for homeowner use. Rather, it is the absence of the restricted use statement that allows for general use. Nothing that can be interpreted as a "general use statement" ever will appear on the product label.

The **physical and chemical hazard statements** identify a given pesticide's flammability or explosiveness. These statements show specific hazards and state conditions to be avoided. For example: "Extremely Flammable;" "Contents Under Pressure;" "Keep away from fire, sparks,

and heated surfaces;" "Do not puncture or incinerate containers;" "Exposure to temperatures above 130º F cause bursting."

The **warranty** information is the manufacturer's assurance that the product conforms to the chemical description on the label and that it is fit for labeled purposes if used according to directions under normal conditions. The warranty does not extend to any use of the product contrary to label instructions, nor does it apply under abnormal conditions such as drought, tornadoes, hurricanes, or excessive rainfall.

Section 5 Use Information

Misuse statements contain language such as, "It is a violation of federal law to use this product inconsistent with its labeling."

Storage and transportation statements may include the following: "Store at temperatures above 32º F;" "Do not contaminate feed, foodstuffs or drinking water;" "Do not store next to feed or food, or transport in or on vehicles containing foodstuffs or feed;" or "For help with any spill, leak fire or exposure involving this material, call Chem Trek (800-424-9300)." Directions for use often comprise the bulk of a pesticide label. They must be adequate to protect the public from fraud and personal injury and to prevent unreasonable adverse effects on the environment. The instructions must provide guidance to the user on the pests controlled, sites of application, compatibility with other pesticides, mixing or dilution rates, application rates, equipment needed for application, timing and frequency of applications, harvest intervals, and general information for successful results.

Directions for use may appear on any portion of the label. Because of the detail required for specific applications, use directions for common sites, pests, and applications may be grouped together under a general heading. Information specific to individual uses may be addressed under specific headings.

Container rinsing and disposal statements list proper procedures for handling pesticide containers and disposing of unused products. Federal, state, and local regulations often must be consulted to determine how to dispose of unused pesticide concentrates or diluted mixtures. Container disposal statements could read "Triple rinse (or equivalent);" "Do not reuse container;" "Offer for recycling or reconditioning;" "Puncture and dispose of in a sanitary landfill;" "Disposal by other procedures allowed by state and local authorities;" "Improper disposal of excess pesticides, spray mixture, or rinsate is a violation of federal law;" "If these wastes cannot be disposed of by use according to label instructions, contact your state pesticide or environmental control agency, or the hazardous waste representative at the nearest EPA regional office for guidance." While numerous pesticide labels still state that properly rinsed containers may be burned, almost every state has clean air laws that prohibit such disposal.

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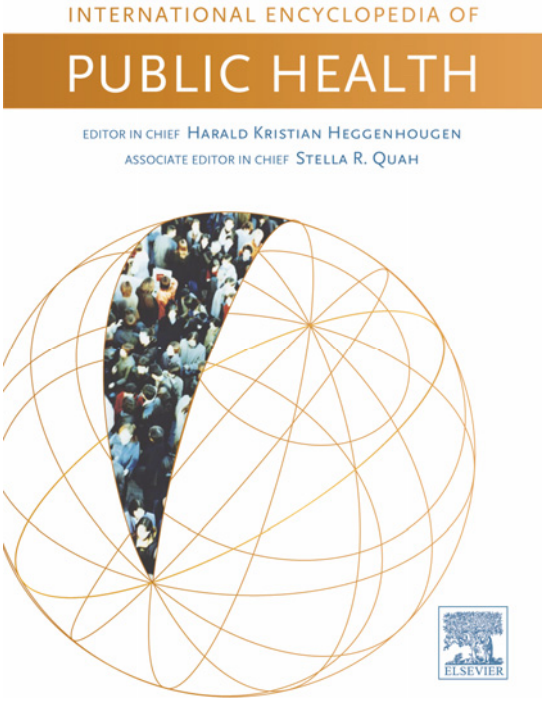

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West Nile Disease

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Introduction

Certain mosquito-borne viruses are known to cause large-scale epidemics, often on a recurrent basis. These agents share epidemiological and ecological features that distinguish them within the 800 recognized arboviruses (arthropod-borne) viruses. Those arboviral infections that may be placed in this epidemiological category of recurrent large-scale outbreaks include St. Louis encephalitis (United States); Venezuelan equine encephalitis (Latin America); Rift Valley fever (Africa and Middle East); chikungunya fever (Africa, south Asia, Southeast Asia); yellow fever (South America, west Africa); dengue fever (North and South America, south Asia, Southeast Asia); Japanese B encephalitis (south Asia, Southeast Asia); Sindbis virus (northern Europe); and western equine encephalitis (western United States). Tens of thousands of human cases may be recorded from each such outbreak, ranging into the hundreds of thousands. The duration of such explosive episodes may be from months to several years, but all characteristically wane for unknown reasons. Many remain locally endemic but at low transmission levels that signify little human risk. The proximal ecological determinants that serve as the basis for the waxing and waning of such outbreaks largely remain undescribed.

West Nile virus (WNV) has long been classified in such an epidemiological category. Since its description in 1937 from a febrile woman in Uganda, WNV has caused large outbreaks with hundreds or thousands of reported cases in Israel (early 1950s and 2000), France (early 1960s), South Africa (1974), Romania (1996), Russia (1999), and the United States (early 2000s). Its enigmatic introduction into the United States in 1999 serves as a reminder of the potential for transcontinental spread of arboviral agents and the public health burden assumed by a country when there is such an event. We seek to review the history of prior WNV outbreaks as well as the current American one; provide a brief overview of the biology of WNV; and describe its mode of perpetuation. In addition, we shall discuss institutional responses historically and more recently; summarize control efforts and outline other modes of intervention; and examine prospects for the future. The American experience with WNV provides public health practice with lessons in responding to new large-scale outbreaks.

History of Epidemics due to WNV

WNV is the most widely distributed arbovirus in the world. The virus was first isolated from a febrile woman in Uganda in 1937, and subsequently was associated with sporadic human cases and infrequent major outbreaks in Africa, Eurasia, Australia, and the Middle East (**Figure 1**). Early studies conducted during the 1950s in Egypt and the upper Nile delta formed the basis for understanding the ecology of the virus. Large epidemics (with hundreds to thousands of cases) occurring prior to 1996 generally affected rural populations, and few cases of severe neurological disease were noted. Beginning in the 1990s, outbreaks began to occur more frequently. These were focused around the Mediterranean Sea and were associated with increases in the frequency of severe disease, including viral encephalitis and persistent neurological sequelae. For example: An outbreak of West Nile disease in and near Bucharest, Romania, in 1996–97 led to more than 500 clinical cases with a case-fatality rate of approximately 10%. Between 1996 and 1999, major WNV epidemics occurred in southern Romania, southern Russia, and the northeastern United States. These outbreaks were notable because they were the first epidemics reported in large urban populations (Zeller and Schuffenecker, 2004). In 2000 in Israel, a countrywide outbreak occurred with a case fatality rate of 8.4%, and neuroinvasive WNV disease was observed in Russia in 2001 and Tunisia in 2003.

The current epizootic epidemic of WNV in North America was the result of a single-point introduction in the New York City area in 1999. The virus has since undergone a dramatic range expansion, and is currently distributed throughout the temperate and tropical Americas (**Figure 2**). The mode of transport and rapid expansion of WNV throughout the Americas remains to be fully understood, although north–south movements clearly are related to bird migratory behavior. This disease was previously unrecognized in the western hemisphere. During 1999–2007 (as of September 25, 2007), 26 282 cases were reported in the United States, of which 1020 (3.9%) were fatal (CDC, 2007a). West Nile disease also has been noted in humans in Cuba and the Cayman Islands, and in equines in Argentina, but scant evidence of human, equine, or avian morbidity and mortality has generally been observed in tropical America, possibly due to cross-protection from other flaviviruses circulating in

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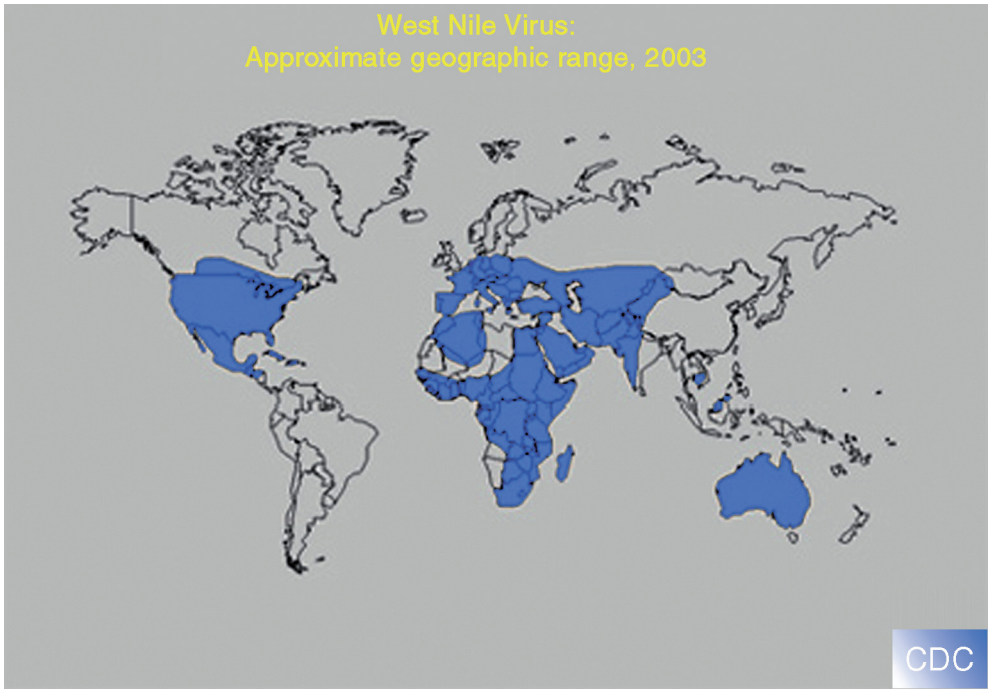

Figure 1 Global distribution of West Nile virus. Reproduced from Centers for Disease Control and Prevention, Atlanta, GA.

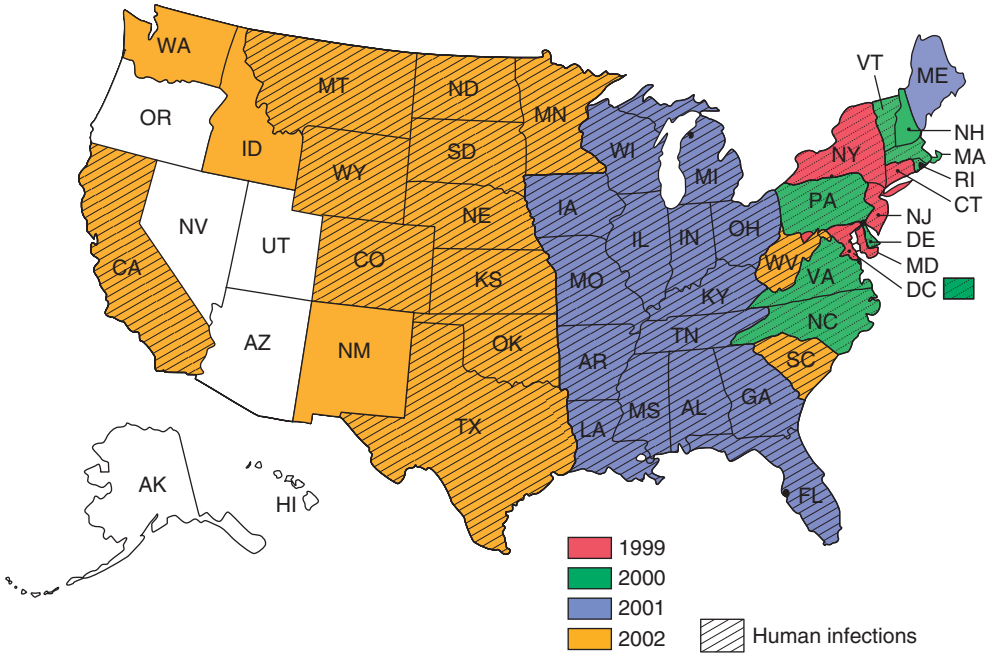

Figure 2 Rapid spread of WNV across the United States. Reproduced from Huhn GD, Sejvar JJ, Montagomery SP, and Dworkin MS (2003) West Nile virus in the United States. *American Family Physician* 68: 653–660.

tropical regions, reduced virulence of WNV in the tropics, or less competent arthropod and avian hosts than in temperate regions in concert with the greater diversity of host species in the tropics. The U.S. Centers for Disease Control and Prevention now recognizes that WNV is permanently established in the Americas, and predicts annual seasonal epidemics into the indefinite future.

There have been no overt cases in the United Kingdom, even with evidence of serological conversions in sentinel chickens. However since 2000, after at least 35 years without disease, WNV has been detected regularly in neighboring France in the Camargue region, with high levels of morbidity in equines. The lack of human cases in northern Europe as compared to southern Europe

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may possibly be attributed to the feeding behavior of local populations of the predominant vector, *Culex pipiens*, as well as yet undescribed other factors.

Biology

West Nile virus is a member of the *Flavivirus* genus of the family *Flaviviridae*, which contains approximately 70 members, most of which are either mosquito- or tick-borne. It is classified within the Japanese encephalitis (JE) serological complex on the basis of cross-neutralization and molecular genetic studies (Calisher *et al.*, 1989). The WNV virion, like other flaviviruses, is enveloped, spherical, approximately 40–60 nm in diameter, and contains an electron-dense core. Mature virions contain a single copy of the positive sense viral RNA packaged within an icosahedral capsid formed by the capsid protein (C). The genome-containing capsid is surrounded by a host-derived lipid bilayer bearing dimers of the viral envelope protein (E) and the membrane protein (M). Following receptor-mediated endocytic entry, membrane fusion, and uncoating, the RNA genome is transcribed as a single long polyprotein that is co- and posttranslationally processed into three structural proteins that form the virus particles and seven nonstructural proteins that replicate the virus genome and interfere with normal host cell function. Thus, the antigenic, genetic, and three-dimensional structure of WNV and its constituent proteins, as well as its replication strategy, are similar to several other flaviviruses, and form the basis for its classification.

The pathogenesis of WNV has been characterized as a balance between virulence, immunity, and viral adaptation (Samuel and Diamond, 2006). Mouse models have been used extensively to characterize the pathogenesis of WNV in vertebrates. After inoculation by mosquitoes, WNV is thought to replicate in Langerhans cells in the skin. These cells migrate to draining lymph nodes and produce a viremia that allows virus seeding of peripheral tissues. Mice that die of WNV appear to suffer severe central nervous system pathology similar to severe human disease. This pathology includes infection of and damage to spinal cord-, hippocampus-, and brain stem-associated neurons. The precise mechanism of WNV neuroinvasion is currently not clear, but appears to be correlated with increased viral load in blood. A wide array of immune effectors are required for protection from lethal WNV infection in mice, including complement, IFN-α/β, $\gamma\delta$ T cells, antibodies, and CD8+ and CD4+ T cells. Moreover, an intact immune system is required for WNV clearance in mice. This is consistent with epidemiological and clinical observations that the immunocompromised and elderly are more likely to suffer severe disease.

The human health burden imposed by WNV may be quite severe. WNV causes 'West Nile fever,' along with more severe illness, including meningitis, encephalitis, acute flaccid paralysis, coma, and death. In humans, approximately 25% of infected persons develop the febrile illness West Nile fever. In fewer than 1%, neuroinvasive illness develops, with clinical symptoms that include headaches, muscle weakness, cognitive difficulty, and polio-like syndrome. Approximately 10% of neuroinvasive cases, or ~0.1% of all infections, terminate fatally (Mostashari *et al.*, 2001). The elderly are significantly more likely to develop neurologic disease; those older than 50 are 10 times more likely, and those 80 years or older are 40 times more likely than those who are younger. Case fatality rates range from 4 to 14% in the recent Romanian, American, and Israeli outbreaks. No specific therapy or vaccine is currently approved for human use. Although heterologous immunity to Japanese B encephalitis (JE) or yellow fever (YF) reduced the severity of disease due to WNV in a hamster model, such an effect was not observed in humans (Johnson *et al.*, 2005), suggesting that the many tens of millions of individuals globally who have been vaccinated against JE or YF would not be protected.

Host Associations

Like other flaviviruses within the Japanese encephalitis antigenic complex, WNV has been isolated from a variety of vectors, but is most commonly associated with *Culex* mosquitoes and particularly the common house mosquito, *C. pipiens*, which is cosmopolitan in distribution. WNV is maintained in nature in an enzootic cycle between ornithophilic mosquitoes and birds (**Figure 3**).

In South Africa, the vector is *C. univittatus*, a highly ornithophilic mosquito that feeds both at the ground and in the canopy. A variety of avian species there including ducks, coot, ibis, egret, doves, and warblers appear to be most important for WNV maintenance. In Israel, WNV appears to be introduced periodically by migrating white storks (Malkinson *et al.*, 2001). Following introduction, it is maintained locally by *C. univitattus*, and has caused extensive outbreaks in domestic geese. *C. univitattus* also is thought to be a major WNV vector in Egypt, although a role for *C. pipiens* has been suggested. In Australia, WNV is maintained by *C. annulirostris* and *C. sitiens* subgroup. During an outbreak in Romania in 1996, *C. pipiens* was implicated as the main arthropod vector, and several bird species, including house sparrows and various species of domestic fowl, had neutralizing antibodies against WNV.

In North America, *C. pipiens* and *C. restuans* maintain WNV in their enzootic cycle in the northeast (Apperson *et al.*, 2004), while *C. quinquefasciatus* and *C. tarsalis* are major vectors in the southern and western United States, respectively. Approximately 317 species of birds have been found infected in North America (CDC, 2007b). WNV has been isolated from large domestic animals

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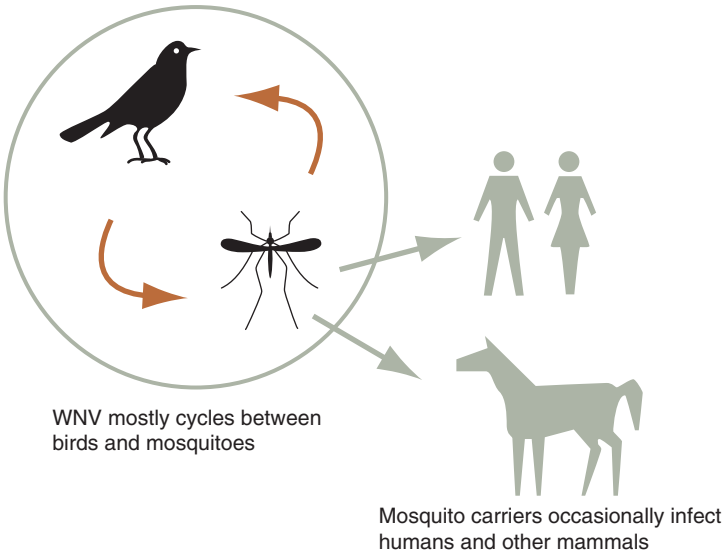

Figure 3 Epornitic cycle of WNV. Reproduced from Metro Vancouver (2004) West Nile Virus. http://www.gvrd.bc.ca/WNV/wnv.htm, with permission from Greater Vancouver Regional District.

(horses, donkeys, goats, buffalo, sheep, pigs, cows), rodents (squirrels, among others), and bats. The number of vertebrate and invertebrate species implicated in WNV transmission is an unusual feature of its ecology that has emerged as its geographic range has spread.

Several aspects of the WNV enzootic transmission cycle require further study. For example, the importance of highly visible species such as crows and other corvids is not well characterized, and may be largely dependent upon site-specific ecological parameters such as mosquito and bird density or overall biome species richness. It is also unclear why the high avian mortality that has been the hallmark of WNV since its introduction into the United States was not observed in the Old World and has not extended to tropical America. Finally, the importance of animals other than birds in WNV transmission continues to be debated. Evidence has been presented that other animals (e.g., some mammals and reptiles) are competent hosts for WNV.

Recent observations have challenged the view that bridge vectors are required for transmitting WNV to humans and other nonavian hosts (Kilpatrick *et al.*, 2006). Typically, a vector-borne zoonotic agent is maintained in nature ('enzootic') by an arthropod with great host specificity feeding on a few species of reservoir host. The zoonotic condition (infection of humans) relies on the participation of an indiscriminately feeding arthropod that bridges the infection from the enzootic cycle to people. Both population-based studies and examinations of host feeding preferences have suggested that *C. pipiens* are responsible for most human infections in the northeastern and north central United States, as well as in eastern Europe and Russia. Early studies by Spielman (1964) hypothesized that there were *C. pipiens* populations that almost never fed on blood (autogenous), some that required blood meals (anautogenous) from birds, and hybrid offspring that formed late in the summer (i.e., in August and later months) that fed indiscriminately on either birds or mammals. It has thus been known for years that *C. pipiens* possess atypical feeding patterns marking two behaviorally and physiologically different forms, autogenous (*C. pipiens* form molestus) and anautogenous (*C. pipiens* form pipiens) populations. *C. pipiens* may therefore be involved in both early season amplification of WNV in enzootic cycles, and as bridge vectors when autogenous–anautogenous hybrids become more common. In support of this observation, a series of field studies has recently shown a strong temporal association between shifts in mosquito feeding behavior from a strong to a weak focus on American robins, with concomitant increases in human disease. Two other significant enzootic vectors in the United States, *C. tarsalis* and *C. nigripalpus*, are similarly important as bridge vectors because of similar shifts in host feeding patterns. Two other species, *C. quinquefasciatus* and *C. salinarius,* feed frequently on both avian and mammalian hosts, including human beings.

The reasons that WNV appears to be increasingly epizootic across large areas of the world remain speculative, but themes seem apparent. Agricultural irrigation was associated with WNV endemicity in the Nile River delta. In Pakistan, irrigation was associated with great densities of *C. tritaeniorhynchus* in a WNV enzootic site. Interestingly, treated wastewater used for agriculture (an increasing practice) created breeding sites for *C. univittatus* in South Africa (McIntosh *et al.*, 1976), and the crops attracted dense infestations of birds, thereby juxtaposing

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the two most important elements of the WNV life cycle. Urban sites infested with pigeons may promote great densities of *C. pipiens*, although pigeons themselves appear irrelevant as reservoirs of WNV. Thus, WNV outbreaks may have a largely anthropogenic basis.

Institutional Responses

WNV serves as the prototype for a rapid institutional response to an emerging infection. Following the definitive identification of the causative agent for the encephalitis cases detected in 1999, investigators from the CDC and New York State undertook comprehensive epizootiologic and epidemiologic investigations in and around the Bronx, New York City, and Long Island. In 2000, the CDC published guidelines for surveillance to assist state and local health agencies and developed ArboNET, an electronic surveillance and reporting system. Annual conferences sponsored by CDC also facilitated a concerted public health response by state and local authorities. Congressional budget appropriations began in 1999, and in particular, $23.8 million was made available through the CDC to states through cooperative agreements. These funds were designated to help state public health departments initiate and carry out surveillance, diagnosis, and prevention of WNV. Such activities included active human case detection, reporting of dead birds, surveys of mosquito infection, public education on personal protective measures against mosquitoes, and serologic and virologic diagnostics. The National Institutes of Allergy and Infectious Diseases of the NIH funded at least 16 grant proposals focusing on WNV during 2000–07, totaling about $4 million, primarily to develop new therapeutics or vaccines. This extraordinary degree of funding support certainly allowed for rapid expansion of the capacity for public health officials to respond and for academic scientists to undertake basic research.

Cases of WNV infection have been acquired from blood transfusion, leading to an FDA mandate to screen all donations for evidence of virus. Diagnostic assays to detect WNV within blood donations were placed on accelerated FDA review and approval. During 2003, 818 infected units were detected from about 6 million donations. A formal cost–benefit analysis of various screening modes suggested, however, that universal screening was not economically justifiable given the overall low risk, but that targeted screening (within high transmission areas and of those units that may be provided to immunocompromised individuals) may have public health utility (Korves *et al.*, 2006).

The urgency with which federal authorities acted requires explanation. From 1933–80, an estimated 10 000 cases of St. Louis encephalitis (SLE) (with 1000 fatalities) occurred in the United States. SLE virus is maintained in a similar epornitic cycle with *Culex* spp. as the maintenance vectors. Indeed, the first human neurologic cases of WNV disease from New York City were first thought to be due to SLE. However, the institutional responses to SLE outbreaks have largely been locally driven, with considerably less available funding. The specter of a foreign disease invading the United States largely influenced the vigorous institutional response, but it seems likely that political pressure from a public alarmed in part by the exotic name of the infection helped drive the federal response. Regardless of motives, the exemplary response to the WNV outbreak strongly suggests that the U.S. infrastructure is capable of effectively responding to other acute infectious disease outbreaks.

Interventions

Surveillance

In 1999, recognition of human cases was presaged by weeks by reports of dead exotic and domestic birds in the New York City area. This observation provided the basis for a cornerstone of WNV surveillance: Reports of dead birds, particularly crows, and the testing of select specimens for evidence of virus, serves as a very sensitive early warning of local transmission, as well as allowing the mapping of transmission activity (CDC, 2003). Use of sentinels such as live captive birds, attempting to document seroconversion, or morbidity/mortality of horses can be useful in rural sites where dead birds are less likely to be detected. Mosquito sampling (particularly larval counts) can provide evidence for trends in the density of important vector species, and testing adult mosquitoes for WNV infection may provide the earliest possible warnings of an outbreak. 'Gravid' traps, in particular, will provide samples with a greater probability of infection than those taken by light traps because *Culex* spp. ovipositing in the fetid water that is used as attractant will by definition have taken a blood meal, whereas light traps will sample mosquitoes that have never fed as well as those that have.

The surveillance case definition (**Table 1**) demonstrates the difficulty with which incidence may be measured. The case definition, in fact, is for arboviral disease in general. Laboratory confirmation can be achieved by direct evidence (isolation of virus or, more commonly, detection of viral RNA by RT-PCR) or by seroconversion. Seroconversion requires the use of specific reagents such as recombinant WNV proteins, inasmuch as there is much cross-reactivity among flaviviruses; even YF vaccination (common for international travelers or immigrants from South America) will induce antibody that may cross-react.

Active case detection for meningoencephalitis serves to better define the public health burden of WNV but plays little role if any in predicting zoonotic risk or need

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Table 1 Surveillance case definition for West Nile virus and other arboviral infections

Clinical description

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis. Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g., paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements)

Laboratory criteria for diagnosis

- Fourfold or greater change in virus-specific serum antibody titer, or
- Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or
- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
- Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition)

Case classification

- Probable: An encephalitis or meningitis case occurring during a period when arboviral transmission is likely and with the following supportive serology: (1) a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or (2) serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen
- Confirmed: An encephalitis or meningitis case that is laboratory-confirmed

Comment

- Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both of these viruses occur
- The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions

Reproduced from http://www.cdc.gov/ncphi/disss/nndss/casedef/arboviral_current.htm.

for intervention. Interestingly, enhanced surveillance for neurologic disease has increased our detection of other arboviral infections endemic to North America such as Powassan encephalitis (tickborne) or Jamestown Canyon virus (mosquitoes). Ensuring that physicians report suspect neurologic cases in real time, as well as requesting the cooperation of diagnostic laboratories in reporting results or forwarding specimens of cerebrospinal fluid for further analysis can help state departments of public health determine the appropriate allocation of resources for predictive surveillance as well as better define the public health burden.

Mosquito Control

When infected mosquitoes are documented, intervention should be considered. Local and state public health boards may differ on when intervention must occur; the choice of a threshold for action is arbitrary but is based on the density and distribution of infected mosquitoes. Two complementary modes of intervention are typical: public education campaigns to promote personal protection (limiting activities at dusk; ensuring that houses have intact window screens; use of repellants when outdoors activity cannot be avoided), and reduction of adult mosquitoes ('adulticiding').

Adulticiding targets those older mosquitoes that have fed at least once, thereby providing the opportunity to have acquired infection, as well as reducing those newly emerged mosquitoes that may feed on hosts that are currently viremic. Misperceptions exist among the lay public regarding aerial or truck-mounted ultra-low-volume (ULV) spraying for mosquito adulticiding, leading to 'environmental' activism that hinders public health efforts. Concerns regarding human exposure, nontarget species kill ("Beneficial insects such as dragonflies that eat mosquitoes also die"), and the limited value of the exercise ("Spraying doesn't work – mosquitoes just come back in a week or so") appear to be mistakenly based on perceptions of agricultural crop dusting, which is a crude approach and one for which such concerns are valid.

ULV spraying for public health intervention utilizes precisely engineered nozzles and atomizers such that the droplet size that is delivered is 50 μm or less, and occurs during dusk or nighttime hours to target flying host-seeking mosquitoes. Wind speed, direction, and temperature are also critically evaluated before undertaking aerial ULV spraying for mosquitoes. The droplet size for agricultural applications ranges from 100–400 μm, which allows delivery in a variety of weather conditions. The precision of aerial ULV spraying for public health purposes

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allow targeting for flying mosquitoes during their activity period, reducing effects on nontarget species (Boyce *et al.*, 2007). The pyrethroid or organophosphate compounds that are used are typically delivered at a rate of 3 oz. per acre, compared with 50–90 oz. per acre for crop-dusting. Thus, adulticiding is a highly targeted action with minimal environmental effect. In fact, mosquitoes quickly reappear within sprayed sites, often within a week or two, demonstrating the absence of lingering environmental toxicity but also confusing the public, who infer that the action was unsuccessful in eradicating mosquitoes. What is unappreciated is that older, infected mosquitoes were targeted and killed; new mosquitoes that emerge subsequent to spraying are less likely to be infected.

The utility of aerial ULV has been questioned with respect to intervening against WNV because the main vectors (*C. pipiens*) in the northern United States tend to be in urban habitats and may be protected by buildings and other infrastructure. However, *C. pipiens* seek out birds in urban tree canopies as well as those roosting on or within buildings and thus would be vulnerable to ULV spraying. In addition, *C. tarsalis*, the main western U.S. vector, and *C. nigripalpis*, an important southern U.S. vector, would be found in habitats amenable to ULV spraying (irrigated agricultural fields for the former; virtually any habitat for the latter).

Preemptive mosquito control may comprise source reduction, in which potential breeding sites are identified and cleaned. For *C. pipiens*, which tends to breed in semi-permanent foul water such as sewers, larvicides such as the growth regulator methoprene or BTI (*Bacillus thuringiensis israelensis*, which secretes a toxin that is active only within the alkaline midguts of mosquitoes) may be effective by delivering it to such bodies of water. Periodic flushing of sewers with large volumes of water may dislodge *C. pipiens* larvae and wash them away: paradoxically, it may be that floods due to heavy rains may actually be detrimental to *C. pipiens* breeding because of this flushing effect.

Personal Protection

Public education about mosquito breeding and biting habits (**Figure 4**) may help to reduce risk at the individual and community levels. Recommendations for avoiding mosquito bites include reducing outside personal activity during dusk and dawn as well as ensuring that screens on windows are intact. Repellants are the main form of personal protection, and a variety of products are commercially available. The CDC has evaluated many such products and generally continues to recommend DEET (diethyltoluamide)-based repellants given their long record of efficacy and general safety record. Rare reports of neurotoxicity, due to overapplication to infants by over-anxious parents, have raised some concerns regarding DEET use, but with tens of millions of applications to humans over the years with only a handful of adverse events, there cannot be any doubt about the public health utility of this repellant. Efficacy is said to be related to DEET concentration, with current recommendations for using formulations containing only up to about 30% active ingredient, which appears nearly as effective as those with greater concentrations; the lower concentrations further reduce the very small risk of an adverse event. DEET needs to be reapplied frequently, depending on formulation. Microencapsulated or liposome-based products tend to control volatilization and provide more extended protection per application.

Vaccines

Vaccine development has been on an accelerated track because of the perceived failure of anti-mosquito interventions in limiting the incidence and geographic spread of WNV. Currently, a successful phase I (safety) clinical trial has been undertaken with a live attenuated chimeric virus based on the yellow fever 17D vaccine (Monath *et al.*, 2006). The YF premembrane and envelope genes have

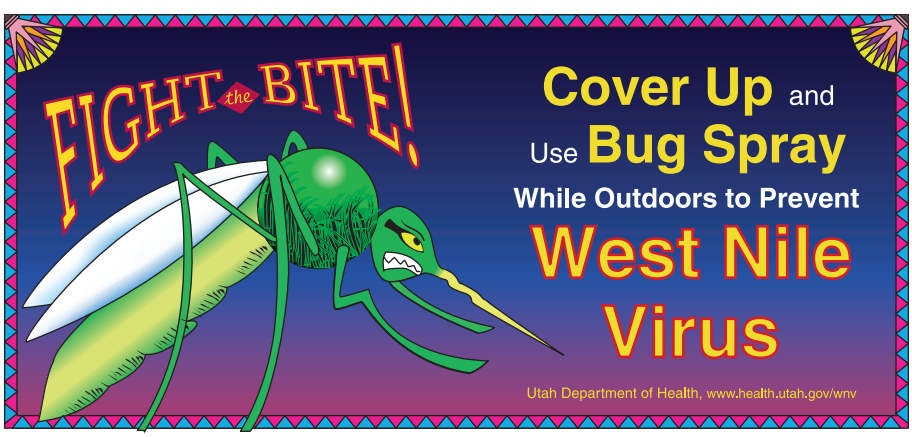

Figure 4 Public awareness poster. Reproduced from Utah.gov, Public Pioneer (2008) West Nile virus returns for '07 season. http://pioneer.utah.gov/internet_resources/features/west_nile.htm, with permission from Utah Department of Health.

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been replaced with those of WNV, with the envelope gene itself being modified to reduce neurovirulence in mouse models. The rapid development of this vaccine candidate reflects the fact that YF 17D is generally recognized as safe, with nearly half a billion persons globally having been vaccinated over its half-century of use. Modifying such a well-known product would facilitate regulatory approval. The phase I trial demonstrated that all of the 45 subjects receiving the chimeric vaccine (Chimerivax WN, Acambis Inc.) developed neutralizing antibody after 1 dose. Neutralizing antibody is critical in protection against arboviral infections.

Effective vaccines for yellow fever, Japanese B encephalitis, and tickborne encephalitis have greatly reduced the public health burden of these flaviviral infections, and it is likely that a WNV vaccine will soon become available. The costs and benefits of its routine use for public health purposes, however, need to be formally stated.

Prospects for the Future

The American experience with WNV has provided a lesson on the lacunae that still exist in responding to explosive outbreaks of vector-borne infection. Although a large pool of well-trained basic researchers and public health workers existed and financial resources were directed to risk reduction and basic research, WNV marched inexorably across the United States from east to west, crossing what appeared to be large geographical barriers in ways that are poorly understood. This east-to-west spread is not readily attributable to transport by birds because few birds undergo migration in such a direction. The distances across which WNV moved are too great to be due to mosquito movements. Without fully understanding the mechanism of such spread, the United States cannot effectively limit the potential for new public health burdens due to other mosquito-transmitted infections that might be introduced as was WNV, such as chikungunya, Rift Valley fever, Japanese encephalitis, Venezuelan equine encephalitis, or dengue. Their eventual arrival into the United States, Europe, or any other continental landmass may be predicted, but institutional capacity to preempt invasion appears to be minimal.

See also: Factors Influencing the Emergence of New (and ''Old'') Diseases; Re-emerging Diseases: Overview.

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Western Europe, Health Systems of

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Background and Context

Europe is a region of approximately 50 independent states, of which 27, largely in the west and center, are now members of the European Union. The concept of 'western Europe' refers loosely to those countries situated to the west of the line that demarcated Soviet and NATO spheres of influence from the end of World War II until 1990. Western Europe includes the countries of Scandinavia (Norway, Sweden, Denmark, and Finland) to the north; Greece, Italy, Spain, and Portugal to the south: the UK and Ireland to the northwest, and France, Germany, Austria, Switzerland, and the Benelux countries (Belgium, the Netherlands, and Luxembourg) in the continental center. This region has a combined population of almost 400 million.

By the end of the 20th century, and in most cases long before, almost all of these people were guaranteed access to health care paid for by a publicly mandated scheme. After pensions, health care is in most of these countries the largest component of public budgets. It is a major source of employment, scientific and industrial innovation, and economic growth. Not least for these reasons, but also because health has such existential significance, health is the stuff of much public debate and political attention (Freeman, 2000).

That European states are also health-care states is the effect of industrialization and of more or less continuous economic prosperity. The health systems of western Europe are the product of democratic politics, liberal and pluralist, in which parties and pressure groups make key contributions to the formulation of public policy. Their development is driven by managed capitalist economies of some kind. They are, at the same time, the realization of a science-based medicine, supported by vigorous and influential pharmaceutical and biotechnology industries.

Health Systems in Western Europe

At the center of the system is the health-care system, founded on scientific biomedicine and consisting of the activity of one or more accredited physicians in relation to disease located in an individual patient body.

This system exists in an uncertain relationship with other complementary or alternative forms of healing, incorporating some and competing with others. These other forms include naturopathy, homeopathy, and chiropractic and are provided in different ways in different countries. They are not usually part of standard welfare entitlements, but are delivered by independent practitioners operating in the marketplace.

Meanwhile, above and beyond (and arguably behind) systems of health care are those of public health, meaning those measures designed to protect the health of the public, which include workplace safety, food standards, health education, and immunization programs, among a range of other services and regulations.

Health-Care Systems

Health systems, as we describe them here, are the formal arrangements by which health care is provided and paid

BATS, ARTIFICIAL ROOSTS, AND MOSQUITO CONTROL

Merlin D. Tuttle, Bat Conservation International

Bats are primary predators of vast numbers of insects that fly at night, and some species consume large numbers of mosquitoes. However, mosquito control is a complex problem that rarely can be solved by a single approach, be it bat houses or pesticides.

A variety of options should be considered, though existing chemical pesticides typically cause more long-term problems than they solve. Chemical poisons kill natural mosquito predators more effectively than mosquitoes. Over time, predators such as fish, insects, and bats, die out while mosquitoes develop resistance, multiplying in ever larger numbers in a losing battle often referred to as "the pesticide treadmill."

Individuals of some bat species can capture from 500 to 1,000 mosquitoes in a single hour[1, 2] and large colonies can consume tremendous quantities. For example, a Florida colony of 30,000 southeastern bats consumes 50 tons of insects annually, including over 15 tons of mosquitoes,[3] and from 77.4% to 84.6% of little brown bats living in the northern U.S. and Canada eat mosquitoes.[4, 5] Because mosquitoes do not take evasive action, and are exceptionally easy to capture, bats may prefer them over larger prey.[6,7] Nursing mothers eat up to their body weight in insects nightly,[8] and often can be attracted to live in bat houses.[9]

However, despite the numbers of mosquitoes that bats eat, simple provision of additional roosts should not be promoted as more than one step in the right direction toward solving mosquito problems. In some cases bat houses may help and in others, they may not. Bats are just one of several groups of animals that naturally prey on mosquitoes. Their relative importance appears to vary from none to high in different locations. In some areas, such as in the far northern tundra or in the Florida Keys, habitats are relatively simple and cannot support more than a few bats or other insectivorous animals, largely precluding natural control. In other locations human activities have converted once diverse biological communities into much simpler farm and yard conditions. Such simple habitats may produce huge hatches of mosquitoes and other insect pests on an occasional basis, while providing insufficient other insect species (such as harmless mayflies) to feed insectivorous animals between hatches of pests. As a result, once-abundant predators that help keep nature in balance are lost. Additional roosts alone may not bring them back.

Although no single approach to mosquito control is appropriate for all locations, encouraging natural predators should be an important element in long-term planning wherever possible. Anything that can be done to encourage predation from aquatic insects, fish, or bats may be important in reducing mosquito numbers.

In some areas, bats may never have been significant predators of mosquitoes, while in others they may have been important. Certainly, in areas like Chautauqua, New York, where bats apparently still play an essential role, all possible precautions should be taken to ensure their continued presence. And where bats are known to have declined, their recovery should be encouraged. Providing additional bat roosts is just one aspect of bat conservation, and saving bats is just one aspect of enhancing natural control of mosquitoes.

It is impossible in most cases, either chemically or naturally, to completely eliminate mosquitoes, though

their numbers can be substantially reduced, and in the long run, this is best done by non-chemical means. Putting up bat houses may help in places where bats can be attracted, but even successful bat houses do not always attract a species that feeds on mosquitoes.

Bat houses are most likely to succeed where bats are already known to use old buildings, barns, or bridges. Such roosting habitat typically is being replaced by structures that are unsuitable for bats, forcing them to find new roosts or die. Participants in the North American Bat House Research Project have attracted thousands of bats to new locations using bat houses, but success is not certain in all locations. In some areas, simply using bat-friendly bridge designs can attract tens or hundreds of thousands of bats. In other places, loss of hibernation caves hundreds of miles away may preclude further use of an area by bat species that feed on mosquitoes. Other species that eat primarily beetles or moths, but do not require caves for hibernation, may still be abundant, with little impact on mosquitoes.

All American bats are beneficial, though their diets vary considerably. While mosquitoes may bother us most directly, many beetles, moths, and other insects consumed by bats are important pests of yards and gardens. Building bat houses may help in many ways, though not necessarily in mosquito control. Plans for most types of artificial roosts are available from Bat Conservation International (BCI), including structures capable of housing from dozens to thousands of bats. Please note that most ready-made bat houses now marketed have nothing to do with BCI and often do not meet minimum standards for success (even though sellers often mention BCI in promotional materials). We do provide a list of other vendors that are BCI-approved (www.batcon.org).

Many kinds of bats have their own unique preferences. Several widespread species will occupy vertical roosting crevices that are three-fourths to one inch wide and about 16 inches deep, though most prefer crevices that are 24 inches deep. Each vertical crevice should end in a horizontal ceiling. Houses are now available that will hold several thousand bats each, though most shelter from 50 to 200.

In cool climates houses need to be painted dark brown or black and receive 7-12 hours of daily sun, while those in hot climates should receive 5-10 hours of daily sun (depending on climate), and be painted medium or light brown, vented, and covered with a tin roof. The latter helps protect from mid-day heat, especially when the roof overhangs the sides. All houses should be painted and sealed tightly with caulk. Only bottoms should be open, except for vents. Locations on buildings or poles 15 to 20 feet above ground are best, preferably near water. In cool climates, bat houses on buildings are the most successful. All inner wood should be at least slightly rough. Many are occupied within 6-12 months, but others require two or more years for bats to move in. For further advice or detailed bat house plans, please call or write to Bat Conservation International, P. O. Box 162603, Austin, TX 78716; phone, 512-327-9721; email, www.batcon.org.

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COMPARISON OF MOSQUITO CONTROL PROVIDED BY THE ARROYO CHUB (*GILA ORCUTTI*) AND THE MOSQUITOFISH (*GAMBUSIA AFFINIS*)

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ABSTRACT. Two 6-wk trials were conducted in 28-m^2 earthen ponds to compare the efficacy of the arroyo chub, *Gila orcutti*, to the mosquitofish, *Gambusia affinis*, as a biological control agent for mosquitoes and a possible replacement for the mosquitofish in sensitive watersheds of southern California. The mosquitofish population growth rate was 1.73 times greater than the arroyo chub population growth rate; however, greater reproduction by the mosquitofish did not result in significantly better reduction of mosquitoes than was provided by the comparatively small populations of arroyo chub. On average across a 6-wk study in the spring, both larvivorous fishes reduced the abundance of 3rd and 4th instars by 4- to 5-fold compared to that observed in the control ponds that lacked fish but contained few invertebrate predators. The abundance of nontarget microinvertebrates in ponds containing the mosquitofish was only 7% of that in ponds containing the arroyo chub during the summer, but did not differ significantly between the fish species treatments when zooplankton was comparatively more abundant during the spring. Even though the number of individuals produced by each fish species during 6 wk in the spring was greater than for fish stocked in the summer, species-specific population growth rates in the spring study (individuals/individual/d; mosquitofish, 0.077; arroyo chub, 0.044) were only slightly higher than in the summer (individuals/individual/d; mosquitofish, 0.068; arroyo chub, 0.039) indicating that differences in the number of fish stocked contributed primarily to the differences in final population size between spring and summer studies. The arroyo chub is native to the South Coastal drainages in California and should be considered as a viable alternative to the mosquitofish for integrated mosquito management programs in riverine wetlands and sensitive watersheds of southern California.

KEY WORDS Larvivorous fish, biological control, *Culex*, native fish, riverine wetlands

INTRODUCTION

The mosquitofish, *Gambusia affinis* (Baird and Girard), can be a useful biological control agent for mosquitoes in particular habitats such as isolated pools, agricultural drainages, and small water bodies in urban environments; however, the mosquitofish may be less desirable for mosquito control than are native fish and insect predators in some natural settings (Moyle 2002). Environmental factors that affect mosquitofish abundance and effectiveness in controlling mosquitoes include the amount of vegetation, abundance of other prey, predators of the mosquitofish, water quality, and factors that regulate mosquitofish breeding cycles (Sawara 1974, Gratz et al. 1996, Swanson et al. 1996). Whereas the mosquitofish can be effective for controlling mosquitoes in nonvegetated or sparsely vegetated man-made impoundments, it is not effective as a mosquito larvivore in all aquatic habitats (Rupp 1996), and the addition of mosquitofish to natural habitats outside its native geographic range is regulated by law or discouraged because the mosquitofish is known to prey upon and competitively eliminate native fishes (Arthington and Lloyd 1989, Courtenay and Meffe 1989, Rupp 1996, Minckley 1999, Sheller et al. 2006). The mosquitofish also consumed the eggs and larvae of stream-dwelling amphibians in experiments carried out in structurally simple laboratory settings where alternative prey were rare (Gamradt and Kats 1996, Goodell and Kats 1999) and may adversely affect amphibian populations in some natural settings (Grubb 1972, Hayes and Jennings 1986; but see Lawler et al. 1999). A substitute for the mosquitofish is needed for settings where local biodiversity may be imperiled by the addition of a nonnative larvivorous fish (Offill and Walton 1999) and for mosquito control in natural settings near encroaching developments.

The arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann), is a good candidate as a biological control agent for mosquitoes in managed and natural wetlands because of its ecology and wide environmental preferences. The arroyo chub is native to the streams of southern California, specifically warm fluctuating streams (Wells and Diana 1975), and has been successfully introduced into several streams outside of its original distribution (Swift et al. 1993). Arroyo chub prefers backwaters that are slow moving and warm (10–24°C), have muddy to sandy bottoms, and are >40 cm deep (Wells and Diana 1975, Bell 1978, Moyle 2002). However, the arroyo chub can be found in other habitats that are adequate for its needs, ranging from reservoirs (Swift et al. 1993) to fast-moving streams (velocities ≥80 cm/s) with coarse bottoms (Bell 1978). The arroyo

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chub is quite tolerant of hypoxia, wide temperature fluctuation (Castleberry and Cech 1986), and fluctuating water conditions (Swift et al. 1993). It is a fractional spawner, breeding somewhat continuously from February through August, but mostly during June and July, in quiet, slow-moving areas where the water temperature is around 14–24°C (Tres 1992).

The arroyo chub is omnivorous; it eats algae, insects, and small crustaceans (Moyle 2002). The arroyo chub prefers eating invertebrates when they are abundant in the spring, but will readily eat algae in the winter when invertebrates are not abundant (Greenfield and Greenfield 1972). The arroyo chub's feeding preferences are somewhat similar to those of the mosquitofish (Swanson et al. 1996), but the arroyo chub is not known to consume amphibian larvae or other fishes. Arroyo chub fry spend 3–4 months feeding on invertebrates in emergent vegetation (Tres 1992), a habitat where mosquito larvae typically occur. It is also known to feed on caddisfly larvae and mollusks in the benthos of cool streams (Richards and Soltz 1986).

Here, the efficacy of the arroyo chub as a mosquito control agent was compared to that of the mosquitofish in 2 studies in earthen ponds in southern California.

MATERIALS AND METHODS

Study site

Two studies were carried out in earthen ponds (4 m × 7 m) at the University of California, Riverside, (UCR) Aquatic Research Facility. A 6-wk study was carried out from June 13 to July 26, 2005. The second study was conducted from April 10 to May 19, 2006. Ponds within 2 rows of 7 ponds per row were used for the studies. Six ponds (3 replicate ponds for each fish species) were used in 2005, and 9 ponds (3 replicates per each fish species and a control without fish) were used in 2006. Offill and Walton (1999) provide a detailed description of the study site. The ponds were devoid of emergent vegetation; 3 cinder blocks were added to the center of each pond to provide refuge for the fishes. Each pond was enriched with 0.65 kg of rabbit pellets (Nutriphase® Rabbit Formula; Pacific Coast Distributing, Inc., Phoenix, AZ) to promote colonization by insects 7 days before stocking fish. Water was supplied through a single pipeline from a reservoir, and water depth was maintained at 0.36 m by float valves.

Physicochemical factors

Water temperatures were measured using maximum-minimum recording thermometers (Markson Scientific, Inc., Del Mar, CA). Water temperatures were recorded every 48–72 h during the duration of each study in 1 northern pond (pond D6) and in 1 southern pond (pond C1). Thermometers were positioned vertically against one of the boards that defined the perimeter of each pond.

Water quality measurements were made 3 wk after the start of each experiment. Specific conductance was measured using an electronic sensor (TDSTester1™; Oakton Instruments, Vernon Hills, IL). Nitrate (in 2005 only) and ammonia concentrations were measured using ion-specific electrodes (nos. 9307 and 9512, respectively; Orion Research, Inc., Beverly, MA). Biochemical Oxygen Demand (BOD_5) in ponds in the 2006 study was measured following American Public Health Association (1995) protocol; an ion-specific electrode (no. 9708; ThermoOrion, Inc., Waltham, MA) was used to measure changes in the dissolved oxygen concentration. Chlorine and pH were measured using a pool water test kit (Taylor Technologies, Inc., Sparks, MD).

Mosquitoes and nontarget invertebrates

Four 350-ml dip samples were taken twice weekly near the corners of each pond to monitor the abundance of mosquitoes and nontarget organisms. The dip samples from each pond were combined using a concentrator cup (mesh opening = 153 μm). Dip samples were taken between 1300 and 1400 h. Specimens were preserved in alcohol (final concentration was approximately 50%). In the laboratory, immature mosquitoes were categorized into 3 subpopulations: 1st and 2nd instars, 3rd and 4th instars, and pupae. Late instars were identified to species using Meyer and Durso (1999).

Nontarget invertebrates were separated into microinvertebrate (zooplankton) and macroinvertebrates (nonculicine aquatic insects). Microinvertebrates were separated into cladocerans, copepods, and ostracods. Macroinvertebrates were keyed to at least the family level using the keys of Merritt and Cummins (1996).

Fish production

Arroyo chubs were collected from a captive population maintained by the Riverside-Corona Resource Conservation District (RCRCD) and transported to a holding pond at the UCR Aquatic Research Facility in spring 2005. The chubs and the offspring produced between the stocking of the holding pond and the beginning of the study were used in the 1st experiment. In 2005, mosquitofish were obtained from the Northwest Mosquito and Vector Control District (Corona, CA). Fish were added to the ponds on June 14.

In 2006, reproductively mature arroyo chub were collected from the RCRCD rearing facility and placed into treatment ponds on April 10, 2006. Mosquitofish were collected from a constructed treatment wetland in San Jacinto, CA (Walton et al. 1998), using minnow traps lined with window screen and baited with dog food. Fish were transported under aeration in coolers, acclimated for >30 min in 50% water from field sites and 50% water from the UCR Aquatic Research Facility, and released into treatment ponds on April 10.

Treatments were assigned to ponds based on larval mosquito densities in pretreatment samples taken on June 13, 2005 or April 7, 2006. In 2005, 2 treatments (mosquitofish or arroyo chub) were distributed among 6 ponds so that the variation in mosquito abundance across treatments was equivalent. Arroyo chubs were stocked into each pond at a rate of 4.5 kg/ha (mean = 12.5 g/pond) as 4 reproductive and 35 larval chubs. Mosquitofish were stocked at 3.4 kg/ha (mean = 9.4 g/pond) as 4 large (approximately 1.25 g/female) gravid reproductive females among 18–25 fish. Because summer 2005 was in the second year of the West Nile virus outbreak in southern California and it was unknown whether the number of human infections would decline as it had done in other regions of the United States, we did not run a treatment without fish. The weight at stocking differed between the two fish species because arroyo chubs are naturally larger than mosquitofish.

In 2006, 3 treatments (mosquitofish, arroyo chub, or control without fish) were replicated in 3 ponds per treatment. Treatments were again distributed among the ponds so that the variation in mosquito abundance across treatments was equivalent. Thirty-one adult arroyo chub were stocked into each of 3 ponds at a rate of 13.2 kg/ha (mean = 37 g/pond). Thirty-one mosquitofish were stocked into each of 3 ponds at 3.6 kg/ha (mean = 10 g/pond). Four large (>1.25 g) gravid *Gambusia* were included in the reproductive individuals added to each pond.

Fish production was estimated at the end of each experiment by collecting fish from each pond using a seine (0.64-cm mesh openings). Three hauls were taken per pond. In 2005, all *G. orcutti* were weighed individually. A random sample of 100 mosquitofish was collected from each pond, and fish were weighed individually. The mosquitofish remaining in collections were weighed together for each pond. In 2006, 100 randomly selected *Gambusia* and *Gila* were weighed individually to construct weight class distributions for each fish species. The remaining adults and juveniles of each species were counted and weighed together by pond. Fish that were not captured with the seine were collected with a dip net after water levels declined and then weighed.

Statistical analysis

Statistical comparisons for the 2005 study were based on natural-log–transformed mean mosquito abundance per dip for each pond using a repeated measures analysis of variance (RM-ANOVA; SYSTAT Version 9.01®; SPSS, Inc., Chicago, IL). Because the variance for the abundance of mosquito pupae was not homogeneous among the treatments, a nonparametric Friedman test was used to test for differences between the 2 treatments in the 2005 experiment. A nonparametric RM-ANOVA on ranks was used to assess the significance of differences for the mosquito larval and pupal subpopulations among the 3 treatments of the 2006 experiment. Pairwise comparisons of mosquito larval abundance between the 3 different treatments was done using a Student-Newman-Keuls test in 2006.

Statistical comparisons for nontarget taxa were based on natural-log–transformed mean abundance of either microinvertebrates (crustacean zooplankton) or macroinvertebrates (nonculicine aquatic insects) using an RM-ANOVA.

The statistical significance of differences of fish production between the treatments containing fish were based on the mean biomass (wet weight) of each species and compared using a *t*-test. A Kolmogorov–Smirnoff test was used to compare weight class distributions of each fish species between the 2 experiments.

RESULTS

Physicochemical factors

Maximum water temperatures of the ponds on the south end of the 2 rows of ponds were approximately 3°C warmer than the northern ponds during summer 2005 (Fig. 1A). The northern ponds were partially shaded, whereas the southern ponds received full sun. The mean maximum and minimum water temperature of the warmer pond, C1, were 32.9°C and 22.6°C, respectively, during the study. The mean maximum temperature in the cooler pond, D6, was 29.6°C, but the mean minimum water temperature (22.8°C) was similar to that in pond C1. The maximum water temperature in pond C1 was 36.7°C during late July.

Unlike the 2005 study, the maximum water temperatures in spring 2006 were similar in the northern (pond D6) and southern (pond C1) ponds (Fig. 1B). The mean maximum water temperatures in the northern pond and southern pond were 28.2°C and 27.5°C, respectively, during spring 2006. The minimum water temperature during the 2006 study was similar in the 2 reference ponds (C1, 18.4°C; D6, 17.7°C). Maximum and minimum water temperatures in-

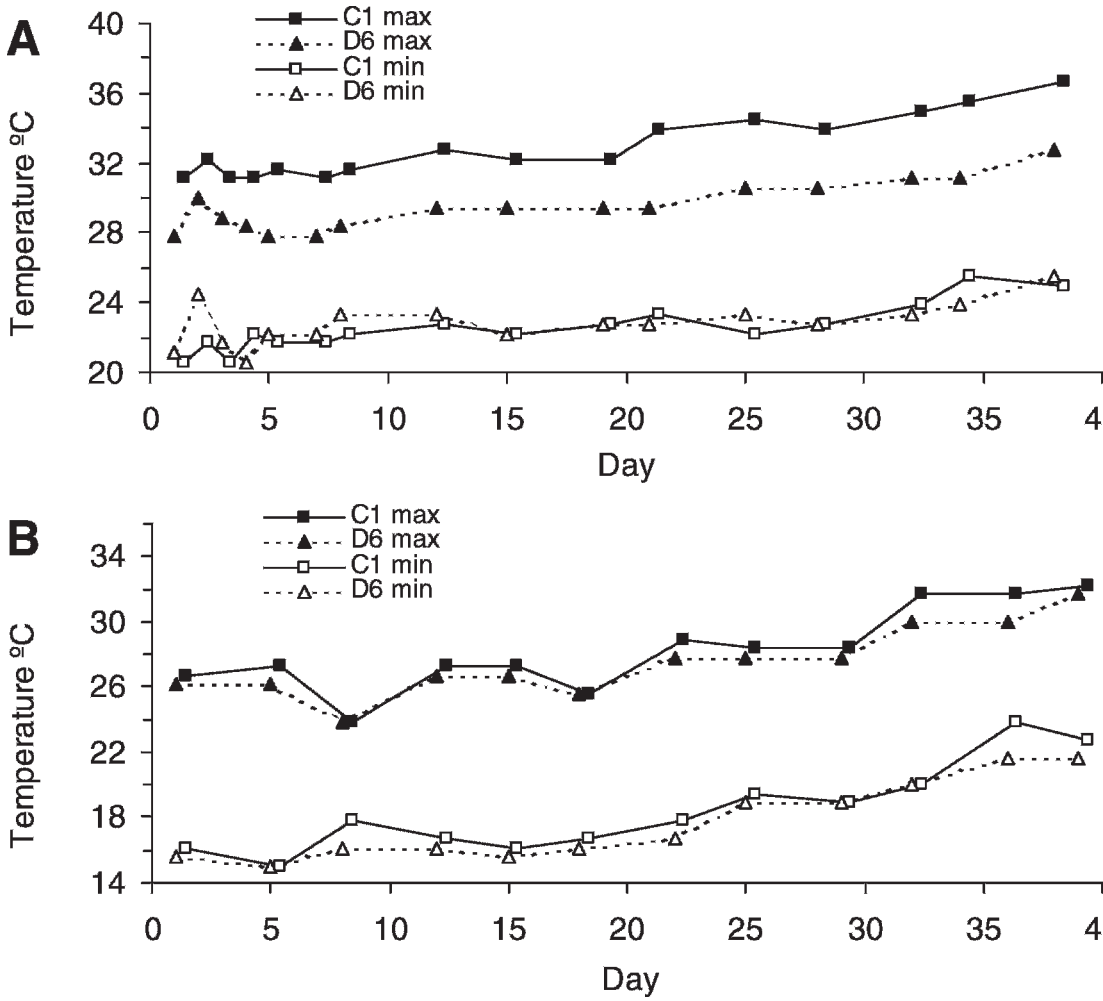

Fig. 1. Maximum and minimum temperatures in 2 ponds at the University of California Aquatic Research Facility, Riverside, CA, (A) from June 13 to July 26, 2005 and (B) from April 7 to May 19, 2006.

creased approximately 5.5°C during the 39-day experiment.

Other physicochemical factors did not differ markedly among ponds in the 2 fish treatments during the 2005 study. Mean (±SE) water conductivity across all of the ponds was 324.3 ± 11.6 μS/cm. The nitrate concentration in ponds with mosquitofish (65.1 ± 6.6 mg/liter) was similar to that in ponds with arroyo chub (60.6 ± 2.3 mg/liter). The chlorine concentration in the ponds was below the limit of detection (0.5 mg/liter). The pH in the ponds was approximately 7.8.

Mean water conductivity across all of the ponds during the 2006 study was 256.7 ± 3.7 μS/cm. The BOD_5 was similar for the 3 treatments (mean ± SE: control ponds 0.73 ± 0.08 mg/liter, arroyo chub ponds 0.76 ± 0.22 mg/liter, and mosquitofish ponds 0.66 ± 0.27 mg/liter). Chlorine concentration (<0.5 mg/liter) and pH (7.8) were consistent across the ponds. Ammonia concentration in the ponds was low (<0.001 mg/liter) and below the level stressful for fish (Swanson et al. 1996).

Mosquitoes and nontarget invertebrates

The predominant mosquito species collected during the 2005 study were *Culex stigmatosoma* Dyar (58% of 3rd and 4th instars) and *Culex tarsalis* Coq. (42% of late instars). Only 1 *Culex erythrothorax* Dyar larva and 1 *Anopheles* sp. larva were collected during the 2005 experiment.

Both fish species provided an equivalent level of control for larval (1st and 2nd instars, RM-ANOVA: F = 0.44, df = 1, 4, $P = 0.55$; 3rd and 4th instars, RM-ANOVA: F = 0.04, df = 1, 4, $P < 0.86$) and pupal (Friedman's test: $\chi^2 = 13.44$, df = 11, $P < 0.265$) mosquito subpopulations. The mean abundance of 1st and 2nd instars in both

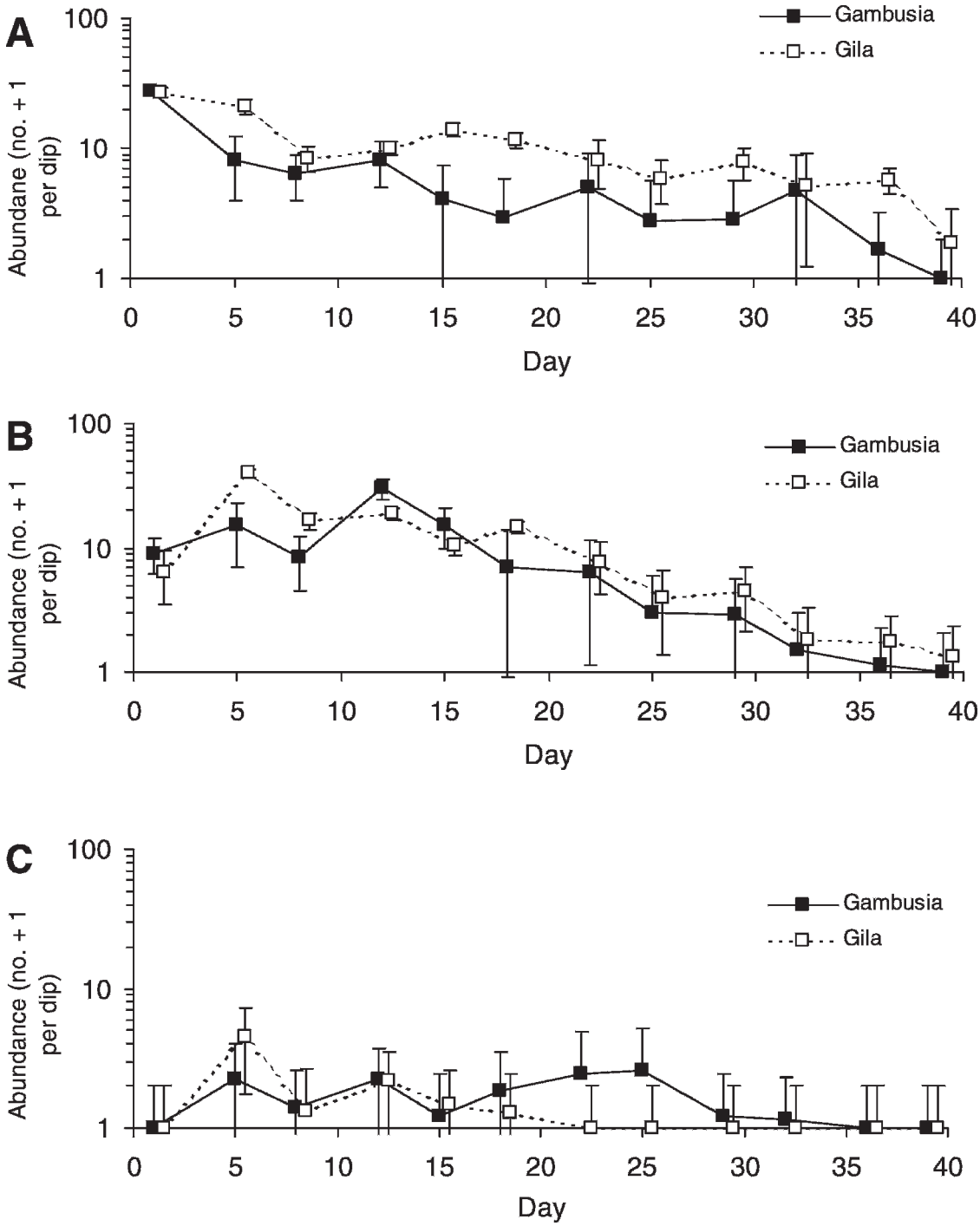

Fig. 2. Abundance (mean ± SE) of mosquito subpopulations in dip samples from ponds containing larvivorous fish during the period from June 13 to July 26, 2005: (A) 1st and 2nd instars, (B) 3rd and 4th instars, (C) pupae. Points are offset horizontally to facilitate illustration.

fish treatments fluctuated at low levels between day 7 and day 36, and then declined to ≤1 larva per dip by day 39 (Fig. 2A). The abundance of 3rd and 4th instars decreased slowly in both treatments after day 12, declining from approximately 20–30 mosquitoes per dip to ≤3 mosquitoes per dip after day 30 (Fig. 2B). Pupae were rarely collected during the experiment (Fig. 2C).

Table 1. Pairwise comparisons for the abundance of immature *Culex* spp. collected in dip samples in 3 treatments during 2006.

Stage[1]	Comparison	Difference of means or ranks	q^2	$P < 0.05$
LI-LII	*Gila orcutti* vs. *Gambusia affinis*	4.00	1.63	No
	G. orcutti vs. Control	15.50	4.69	Yes
	G. affinis vs. Control	11.50	4.47	Yes
LIII-LIV	*G. orcutti* vs. *G. affinis*	3.50	1.43	No
	G. orcutti vs. Control	11.00	4.49	Yes
	G. affinis vs. Control	14.50	4.19	Yes

[1] L, larval instar.
[2] Student-Newman-Keuls statistic.

In 2006, the predominant mosquito species collected was *Cx. tarsalis* (mean relative abundance 99%). Only a few *Cx. stigmatosoma* larvae were collected.

Both fish species significantly reduced the abundance of larval mosquitoes (1st and 2nd instars: $\chi^2 = 11.021$, df = 2, $P < 0.004$; 3rd and 4th instars: $\chi^2 = 10.178$, df = 2, $P < 0.006$) compared to mosquito subpopulations in the control ponds without fish during spring 2006 (Table 1). First and second instars in the control ponds increased from ≤1 larva per dip on day 15, to nearly 15 larvae per dip on day 25 (Fig. 3A). The abundance of early instars in the ponds containing arroyo chub and mosquitofish was similar to that in the control ponds through day 15 but then remained at levels lower than the control ponds until the end of the experiment (Fig. 3A).

The abundance of *Culex* spp. 3rd and 4th instars in the control ponds was similar to the

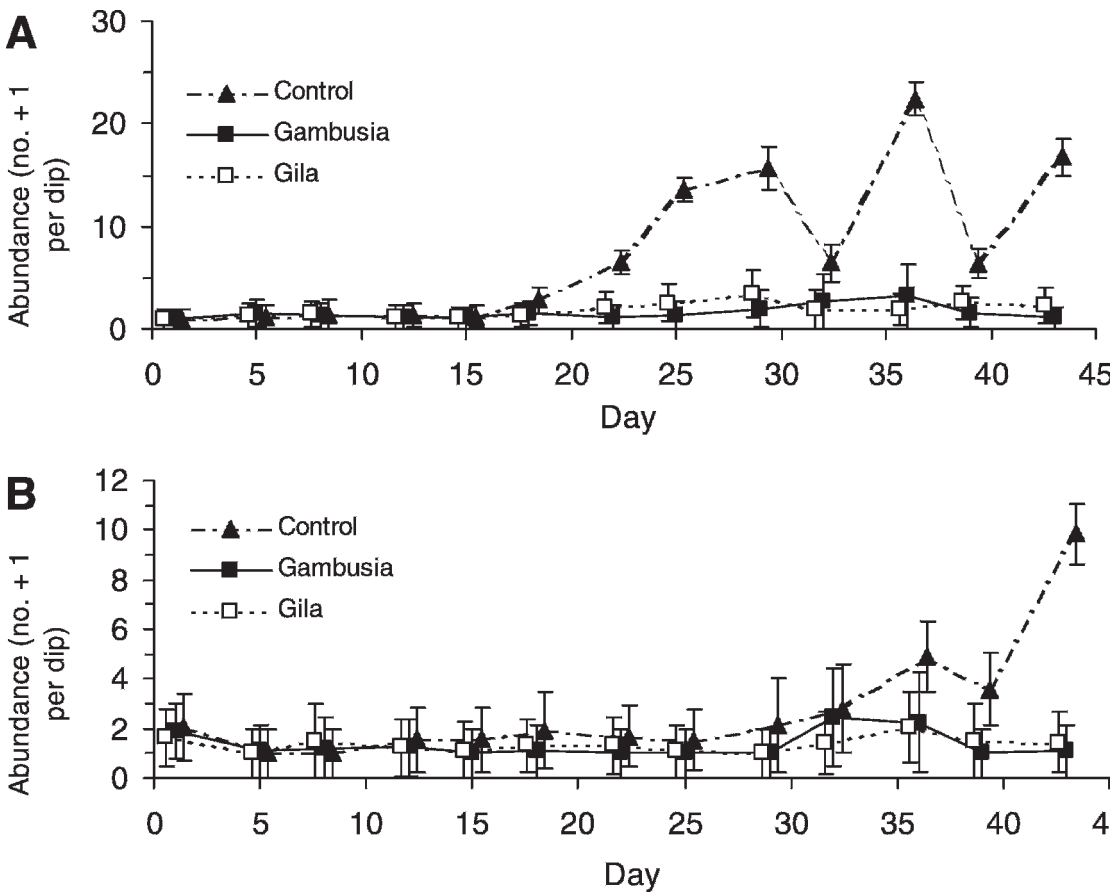

Fig. 3. Abundance (mean ± SE) of *Culex* spp. immature subpopulations from 3 treatments during the period from April 7, 2005 to May 19, 2006: (A) 1st and 2nd instars and (B) 3rd and 4th instars. Points are offset horizontally to facilitate illustration.

Table 2. Mean abundance (± SE) of nontarget invertebrates collected in dip samples among ponds containing and lacking larvivorous fish treatments during 2 experiments.

Nontarget group	Treatment			P [1]
	Gambusia affinis	*Gila orcutti*	Control (no fish)	
2005 experiment				
Microinvertebrates	3.87 ± 1.75	53.99 ± 14.62		0.029
Macroinvertebrates	9.10 ± 4.07	9.06 ± 3.31		0.886
2006 experiment				
Microinvertebrates	244.43 ± 95.17	281.80 ± 31.97	201.53 ± 24.55	0.643
Macroinvertebrates	8.20 ± 1.51	9.50 ± 1.86	10.28 ± 1.59	0.515

[1] P-values from repeated measures analysis of variance.

ponds containing *G. orcutti* and *G. affinis* for about 1 month and then was higher than in the ponds containing fish. After day 32, late instars in the control ponds increased to 9 larvae per dip by the end of the experiment (Fig. 3B). The numbers of *Culex* 3rd and 4th instar collected from mosquitofish ponds and arroyo chub ponds remained at comparatively low levels (≤1 larva per dip) through day 43.

The abundance of mosquito pupae did not differ significantly among the 3 treatments during the 2006 study ($\chi^2 = 0.087$, df = 2, $P < 0.957$). After day 5, the abundance of *Culex* pupae was low throughout the experiment, except after day 35 in the ponds without fish, where the abundance of pupae increased as the comparatively large populations of larvae completed development.

The numbers of nontarget microinvertebrates in dipper samples differed significantly between the ponds with mosquitofish or arroyo chub during the 2005 experiment (Table 2). The abundance of microinvertebrates in the ponds stocked with the former species after day 8 was significantly lower than in ponds stocked with the latter (Fig. 4A). Microinvertebrates in ponds containing arroyo chubs were approximately 14 times more abundant than in the ponds containing mosquitofish (Table 2). The groups of macroinvertebrates collected were mostly rare (Table 3) and the abundance of nontarget insects did not differ significantly between the 2 fish treatments (Table 2).

In 2006, there were no significant differences in the numbers of nontarget microinvertebrates and macroinvertebrates in dipper samples among the 3 treatments (RM-ANOVAs, Table 2). Microinvertebrate abundance during 2006 (Fig. 4B) was greater than during 2005 and, unlike the 2005 study, did not differ significantly between ponds stocked with mosquitofish or arroyo chub. The most common nontarget invertebrates were again the microinvertebrates (Table 3). The other invertebrate groups were comparatively rare in 2006.

Fish production

There was no statistical difference in the weight class distributions for each fish species between the 2005 and 2006 studies (Kolmogorov–Smirnoff pairwise comparison, $P > 0.05$). The majority of individuals of both fish species weighed ≤1 g at the end of both studies (data from both studies combined: Figs. 5A, 5B).

Production of the 2 fish species differed significantly in 2005 (t-test, $P < 0.048$). The mean wet weight of individual mosquitofish was 0.43 ± 0.03 g at the end of the 2005 study. On average, each arroyo chub (0.64 ± 0.05 g) was 50% heavier than was an individual mosquitofish. At the end of the 2005 experiment, many more mosquitofish were produced per pond, and biomass after 6 wk (57.8 kg/ha; 161.73 ± 34.30 g/pond) was 12.4 times higher than for *Gila* (4.6 kg/ha; 13.00 ± 2.90 g/pond; Fig. 6A). Mosquitofish biomass increased 3.90 g/day and *G.* orcutti biomass increased only 0.10 g/day.

In 2006, a similar number of fish, rather than a roughly equivalent biomass, was added to ponds, and fish biomass after 6 wk did not differ significantly between the 2 fish treatments (t-test, $P < 0.990$). Most of the fish collected at the end of the experiment were small (mean ± SD: mosquitofish, 0.12 ± 0.02 g; arroyo chub, 0.46 ± 0.12 g) but, on average, each arroyo chub was nearly 3 times heavier than each mosquitofish. Fish biomass at the end of the experiment was >30 kg/ha per pond (mosquitofish: 33.14 kg/ha, 92.78 ± 15.66 g/pond; arroyo chub: 32.99 kg/ha, 92.37 ± 15.80 g/pond; Fig. 6B). During spring 2006, biomass (wet mass) increased 2.12 g/day for mosquitofish and 1.41 g/day for arroyo chub.

Mosquitofish produced 19 times and 4 times more offspring than did arroyo chub in the summer and spring, respectively. Based on calculations using the total fish biomass per pond and the mean mass per individual for each study, the mean number of mosquitofish per pond was estimated to be 376 individuals (range based on the SE: 296–456) and 773 individuals (range: 643–904) at the end of the summer and spring studies, respectively.

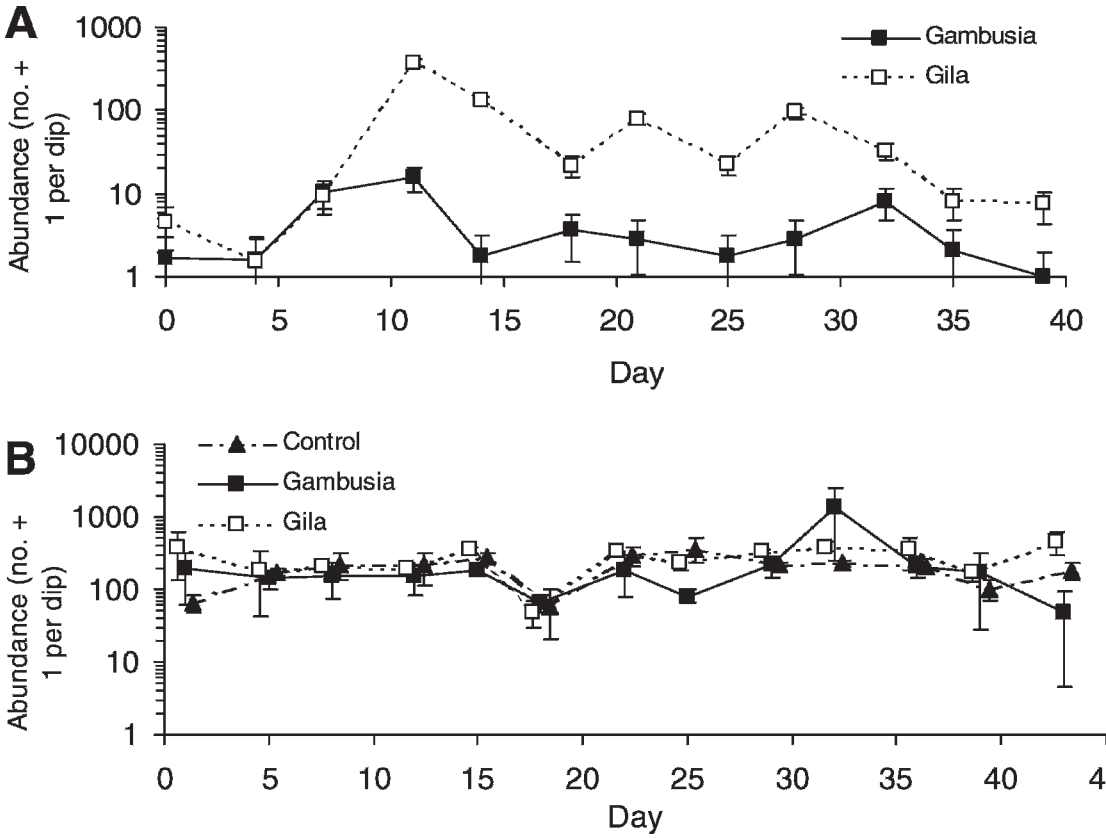

Fig. 4. Microinvertebrate abundance per dip sample (mean ± SE) taken (A) from June 13 to July 26, 2005, and (B) from April 7, 2005 to May 19, 2006. Points are offset horizontally to facilitate illustration.

Table 3. Nontarget taxa collected from experimental ponds at the University of California Aquatic Research Facility in Riverside, CA, from June 13 through July 22, 2005, and from April 10 through May 19, 2006.

Nontarget group	Abundance[1] 2005	Abundance[1] 2006
Anisoptera: Aeshnidae	R	R
Anisoptera: Libellulidae	R	R
Ceratopogonidae	R	R
Chironomidae	C	C
Cladocera	C	A
Copepoda	C	A
Corixidae	R	R
Dytiscid larvae	R	U
Ephemeroptera	R	R
Ephydrid larvae	R	R
Hydrophilid larvae	R	R
Laccophilus spp.	R	R
Notonectidae	R	R
Ostracoda	R	R
Veliidae	R	R
Zygoptera: Coenagrionidae	R	R

[1] A, abundant (≥10,000 individuals collected); C, common (1,000 ≤ C < 10,000 individuals collected); U, uncommon (100 ≤ U < 1,000 individuals collected); R, rare (< 100 individuals collected).

The mean number of arroyo chub per pond was 20 individuals (range: 15–25) and was estimated to be 200 individuals (range: 166–235) at the end of the summer and spring studies, respectively.

The species-specific population growth rate was similar in both studies, albeit populations of both fish species were growing slightly faster in the spring vs. the summer. If it is assumed that the fish populations were growing exponentially in the ponds during the 6 wk after stocking, then the intrinsic rate of increase of *G. affinis* was 1.73 times that for *G. orcutti*. The rate of population increase for the mosquitofish was 0.068 (range based on SD: 0.056–0.080) individuals · individual^{-1} · day^{-1} during summer 2005 and 0.076 (0.069–0.083) individuals · individual^{-1} · day^{-1} during spring 2006. The rate of population increase for the arroyo chub was 0.039 (0.038–0.040) individuals · individual^{-1} · day^{-1} during the summer study and 0.044 (0.037–0.051) individuals · individual^{-1} · day^{-1} during the spring study.

DISCUSSION

The mosquitofish and the arroyo chub provided equivalent levels of mosquito control in

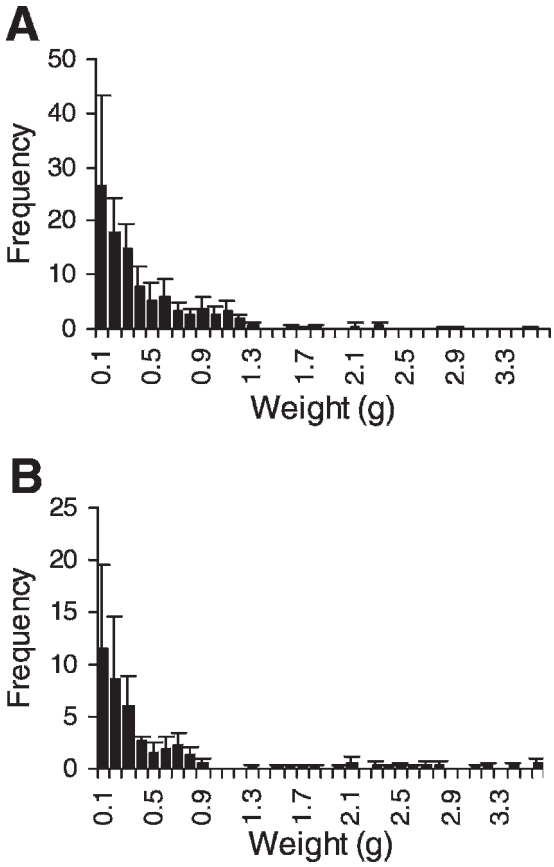

Fig. 5. Relative abundance of weight (wet weight) classes of 2 larvivorous fishes (A, *Gambusia affinis*; B, *Gila orcutti*) from the 2005 and 2006 experiments at the University of California Aquatic Research Facility, Riverside, CA. The mean ± SD is illustrated for each weight category.

earthen ponds during two 6-wk studies. The mosquitofish was more prolific than the arroyo chub; however, greater reproduction by mosquitofish did not result in significantly better reduction of mosquitoes than was provided by the comparatively smaller populations of arroyo chub. Both larvivorous fishes provided better mosquito control than did the small numbers of invertebrate predators in the control ponds that lacked fish. High water temperatures (>33°C) during a previous study at this site significantly reduced the survival of the threespine stickleback (*Gasterosteus aculeatus* L.), an alternative larvivorous fish to mosquitofish (Offill and Walton 1999), but both fish species used in the current study survived high water temperatures without significant mortality.

Hot weather, which decreased mosquito ovipositional activity at the site (Van Dam 2007), and density-dependent predation pressure on immature mosquitoes from the larvivorous fish populations were probably important factors contributing to the decline in mosquito populations observed in July 2005. A steep drop in the abundance of early instars in the ponds stocked with mosquitofish occurred during the last 3 sampling dates of the 2005 study. After day 10 of the 2005 experiment, microinvertebrates in ponds stocked with mosquitofish were 14-fold less abundant than in the ponds with arroyo chub, suggesting that the former species was consuming microinvertebrates preferentially to the immature mosquitoes. The larger number of young mosquitofish compared to arroyo chub may explain the more rapid decline in the early-stage larval mosquito populations in ponds stocked with mosquitofish. Immature-mosquito abundance also decreased in the ponds stocked with arroyo chub, although the decline in numbers was not as rapid as in the mosquitofish ponds. When alternative prey, such as Cladocera, is abundant, small mosquitofish preferentially feed upon zooplankton (Bence 1988). Furthermore, large mosquitofish prefer feeding on predatory insects compared to mosquito larvae (Bence and Murdoch 1986, Bence 1988). Therefore, when a mosquitofish population is at an intermediate level of buildup after stocking and alternative prey are abundant, larvivorous mosquitofish may not reduce immature mosquito abundance significantly (Bence 1988). In 2005, it is likely that the natural attenuation of the mosquito population after initial flooding, the initially small larvivorous fish population and abundant alternative prey, and an initially prolific mosquito population resulted in a low level of mosquito control and the gradual decline in the mosquito population seen in both treatments

In contrast to the 2005 study, mosquito populations did not decline continuously across the 2006 experiment in the treatments with larvivorous fishes. The resurgence of mosquito populations in control ponds after 2 wk was likely attributable to the vernal peak period of *Culex* oviposition. The abundance of *Culex* early instars in the fishless control ponds increased appreciably after day 15, indicating an intensification of mosquito ovipositional activity that, relative to the initial 2 wk of the study, remained high for the next 4 wk of the study. Water quality variables (ammonia, BOD_5, chlorine, nitrate, pH, specific conductance) in the ponds were equivalent across the 3 treatments in 2006 and did not contribute to differences of mosquito abundance between the treatments containing or lacking larvivorous fish. Van Dam (2007) found that egg laying by *Cx. tarsalis* on water conditioned by mosquitofish was lower than on aged tap water alone in the laboratory. This might partially explain the differences of mosquito abundance observed in the ponds containing fish vs. lacking fish in 2006.

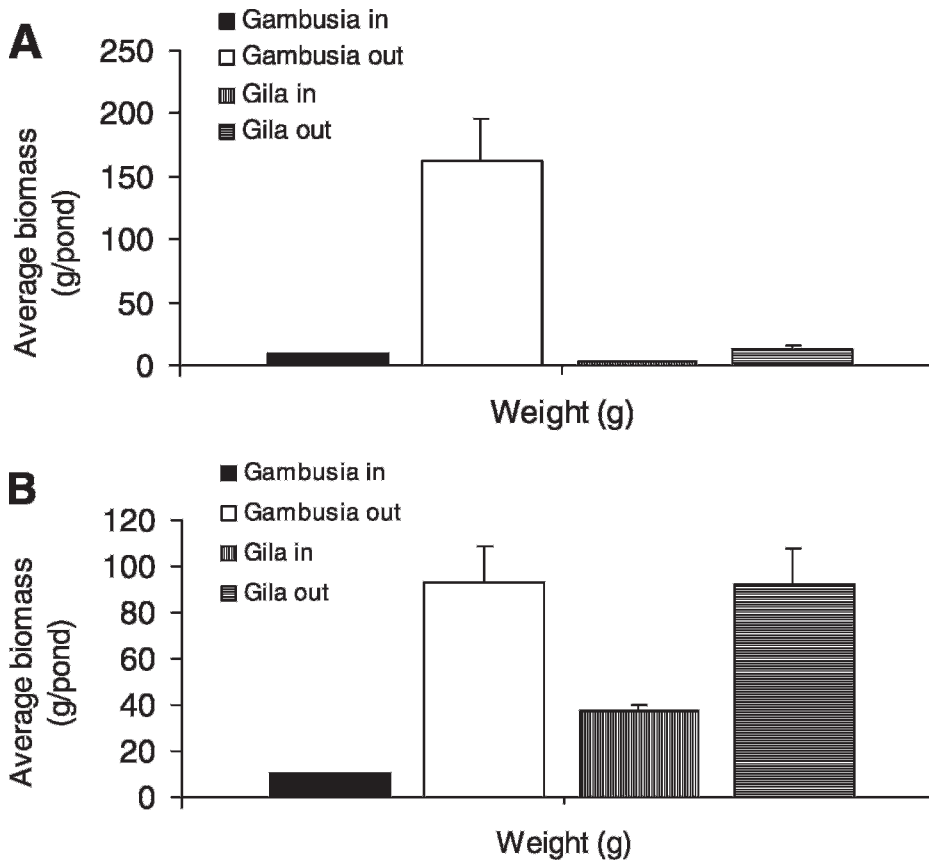

Fig. 6. Fish biomass at stocking and after 6 wk for 2 studies at the University of California Aquatic Research Facility, Riverside, CA, (A) 2005 experiment and (B) 2006 experiment.

Microinvertebrate abundance did not differ among the 3 treatments during spring 2006, unlike the 2005 study, in which microinvertebrate abundance in ponds stocked with *Gambusia* was on average 7% of that in the ponds stocked with arroyo chub. Yet, there was a steep decline in the number of microinvertebrates in ponds containing mosquitofish during the last week of the 2006 experiment. Microinvertebrate abundance during the 2006 experiment was much higher compared to the 2005 study (cf. Figs. 4A, 4B) and may have played a role in dampening the effect of *Gambusia* predation on microinvertebrates until the end of the experiment when *Gambusia* were at their highest density per pond.

Biomass production for each fish species differed between treatments and years. Mosquitofish production during 6 wk in summer 2005 was nearly twice that recorded for a similar period in spring 2006 (final mean biomass: 57.8 g/ha vs. 33.1 g/ha). The lower mosquitofish production during spring 2006 was perhaps caused by the direct effect of cooler spring temperatures on mass-specific growth rates. While the mosquitofish population size in the 2006 study was greater than in 2005, the abundance of alternative prey (i.e., zooplankton) was nearly 60-fold that in the 2005 experiment; therefore, strong intraspecific competitive interactions were not likely to have caused the lower mass-specific production during spring 2006.

Arroyo chub production showed a trend opposite to that of mosquitofish, with greater production during the spring than in the summer. The addition of a greater number of adult fish in spring 2006 than in summer 2005 (31 vs. 4) contributed to the differences in production between years. In 2006, the larger number of adult arroyo chub reproduced and biomass in ponds increased more than 7-fold (33 kg/ha vs. 4.6 kg/ha) compared to 2005.

Even though the number of individuals produced by each fish species differed between the 2 studies, the species-specific population growth rate was similar in both studies, indicating that differences in the stocked populations contributed primarily to the differences of population size between spring and summer studies. The ability of arroyo chub to increase to sufficient numbers to control mosquitoes is linked to the time of the

year of stocking, the reproductive status of the stocked fish, and the number of fish stocked. This study indicated that stocking the arroyo chub in the spring resulted in greater production than stocking during the summer, and given the 3-fold difference in wet mass between the adults of the two fish species, the mass-specific stocking rate for arroyo chub is higher than for the mosquitofish. Various studies have suggested different spring stocking levels for mosquitofish ranging between 1 and 4 kg/ha (Kramer et al. 1988, Walton and Mulla 1991, Offill and Walton 1999). For mosquitofish, it is critical to stock fish in the early spring because winter die-off can significantly reduce mosquitofish populations (Walton 2007). The arroyo chub should be stocked in early spring (March or early April) at the beginning of their annual reproductive period (Tres 1992), and an area-specific mass >10 kg/ha may be required if mosquito control is needed soon after stocking fish; further studies are needed.

Our studies suggest that native arroyo chub may be a viable replacement for nonnative mosquitofish in natural aquatic ecosystems in the South Coastal drainage system of southern California, especially in riverine wetlands used to improve water quality and provide habitat for endangered species. The arroyo chub provided levels of mosquito control similar to that provided by the mosquitofish and did not significantly reduce nontarget invertebrate populations during both 6-wk studies. Unlike the stickleback, which also has been considered as an alternative larvivorous fish to the mosquitofish, the arroyo chub is capable of withstanding high summer water temperatures. The arroyo chub is likely to provide meaningful levels of mosquito control in wetlands associated with rivers and streams in southern California because the arroyo chub is better adapted to life in slow and fast moving water than is the mosquitofish. Movement of fish among watersheds is often restricted because of genetic differences among populations, potential transfer of pathogens and parasites, and the presence of other sensitive species; therefore, coordination of vector control activities using the arroyo chub will require coordination with federal and state agencies responsible for the management of natural resources. Further studies are needed to assess the sustainability of chub populations and the efficacy of the arroyo chub for mosquito control in wetlands.

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LARVIVOROUS FISH INCLUDING *GAMBUSIA*

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KEY WORDS *Gambusia*, mosquitofish, culture, stocking

INTRODUCTION

Mosquitofish [*Gambusia affinis* (Baird and Girard) and *G. holbrooki* Girard] have been used as a biological control agent for mosquitoes for more than 100 years and, when used under appropriate conditions, remain one of the best candidates for biological control programs against mosquitoes. Mosquitofish are prolific, hardy, omnivores that are capable of rapidly producing large populations following introduction into most aquatic habitats. During the 20th century, *Gambusia* was introduced to more than 60 countries on nearly every continent and the 2 mosquitofish species remain the preferred larvivorous fishes (85–90% of studies) for biological control programs focused on mosquitoes (Gerberich and Laird 1985).

The literature on mosquitofish is vast and numerous studies on native fishes as replacements for the mosquitofish in biological control programs have been carried out since Meisch's (1985) and Bay's (1985) reviews of larvivorous fishes previously used in mosquito control programs. Recent bibliographies (Haas and Pal 1984, Gerberich and Laird 1985, Ahmed et al. 1988, Legner 1995), as well as a recent publication focusing on mosquitofish culture and use in mosquito control programs (Swanson et al. 1996), illustrate the great interest and integral role that larvivorous fish fulfill in modern mosquito control programs. Ahmed et al. (1988) compiled a bibliography of 204 references related to indigenous fishes for mosquito control and 96 references for fishes for aquatic weed control. Gerberich and Laird's (1985) review of the literature on larvivorous fishes found that more than 253 fish species have been considered for the biocontrol of mosquitoes.

The great interest in *Gambusia* as a control agent for mosquitoes is tempered by the concerns of icthyologists and ecologists for the potential negative aspects of mosquitofish on non-target organisms and natural ecosystems (Arthington and Lloyd 1989, Meffe and Snelson 1989, Rupp 1996, Gratz et al. 1996). Regardless whether a particular fish species is used in biological control programs or stocked for recreational fisheries, adding non-native fish to ecosystems can have dramatic consequences on the fauna of some aquatic habitats (Goodman 1991, Adams et al. 2003), especially in habitats lacking fish (Wellborn et al. 1996, Hamer et al. 2002). A greater awareness of the ecological consequences of biomanipulation is needed (Rupp 1996) including both the direct effects of predation on non-target taxa and indirect trophic effects such as facilitation of other non-native organisms (Stachowicz 2001, Adams et al. 2003).

This chapter provides an overview and highlights some of the important advances in our knowledge of mosquitofish and other larvivorous fishes since Meisch's (1985) and Bay's (1985) reviews, respectively. The reader is referred to Swanson et al. (1996) for thorough discussion of mosquitofish biology, culture systems and use of *Gambusia* spp. as a biological control agent for mosquitoes, and to Gerberich and Laird (1985), Ahmed et al. (1988), Legner (1995) and Hurst (2004) for bibliographies of larvivorous fishes used for mosquito control.

GAMBUSIA AFFINIS *AND* G. HOLBROOKI

BIOLOGY

The 2 mosquitofish subspecies recognized in 1985 have been elevated to species. Based on the original distributions (Rosen and Bailey 1963) and morphological (Rivas 1963), chromosomal (Black and Howell 1979), and biochemical differences, Wooten et al. (1988) distinguished the western mosquitofish, *Gambusia affinis* (Baird and Girard), from the eastern mosquitofish, *G. holbrooki* Girard. Both species are native to the southeastern United States (Rauchenberger 1989). In North America, the original range (circa 1900 before widespread stocking for mosquito control) of *G. affinis* extended from southern Illinois and Indiana to Alabama, Texas, and northern Veracruz, Mexico. *Gambusia holbrooki* was found from New Jersey to Florida and southern Alabama. During the last 100 years, both species have been utilized worldwide for mosquito control and currently occur on every continent except Antarctica and have been introduced throughout much of the IndoPacific region (Swanson et al. 1996).

Mosquitofish are sexually dimorphic (Fig. 1). Mature males [<35 mm or 1.4 in. total length (TL)] are smaller than mature females (<60 mm

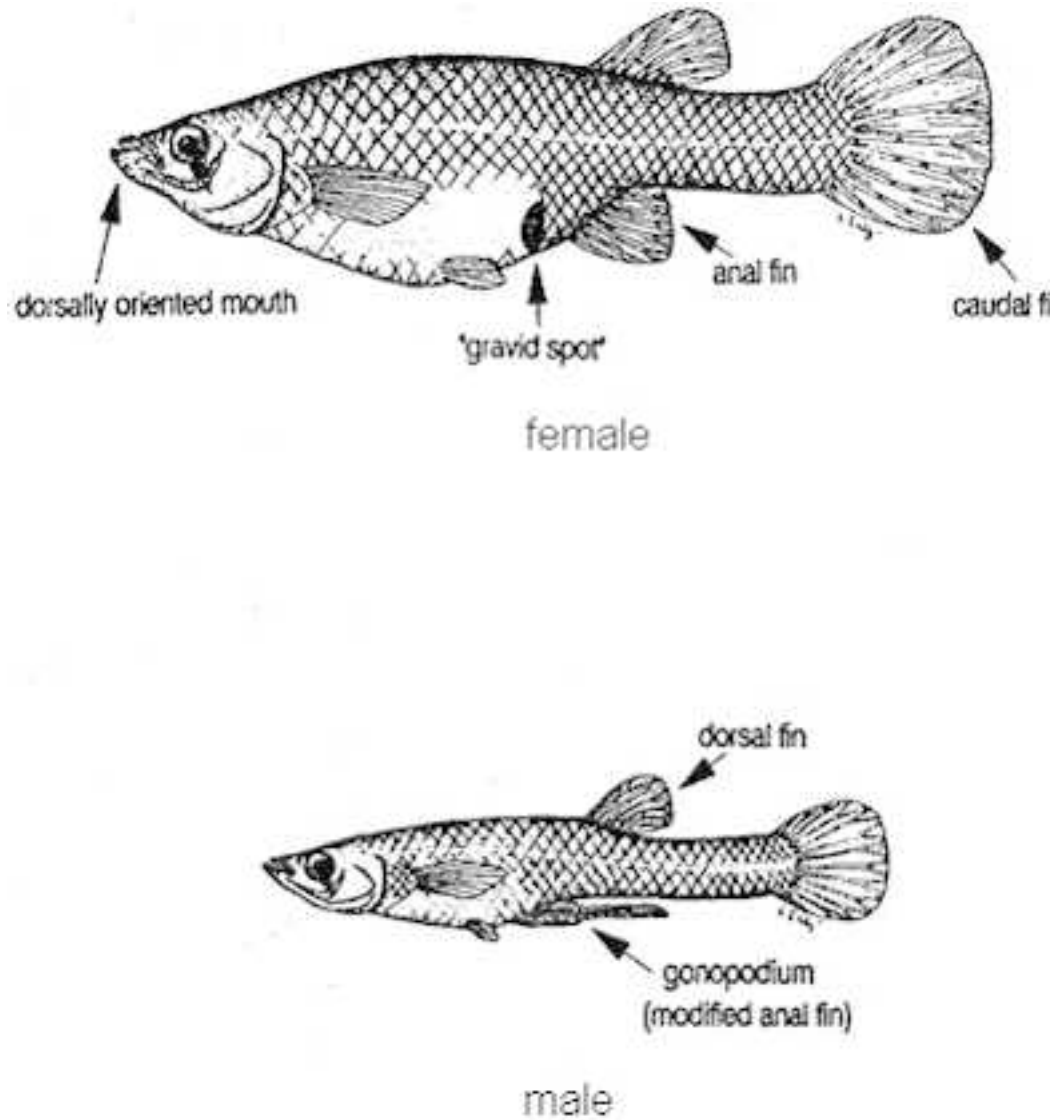

Fig. 1. Male and female mosquitofish (from Coykendall 1980).

or 2.4 in. TL), have a comparatively slender body shape and have a gonopodium, a modified anal fin used as an intromittent organ (Moyle 2002). Unlike the growth of males that stops after reaching sexual maturity (but see Puigcerver 1992), growth in female mosquitofish is indeterminate (Vondracek et al. 1988, Cabral and Marques 1999). Growth of females slows after sexual maturation when resources are invested mostly into reproduction (Krumholz 1948, Hubbs 1971).

The morphology of the male intromittent organ has been used to distinguish *G. affinis* from *G. holbrooki* (Rivas 1963, Rauchenberger 1989). In her cladistic analysis of the genus *Gambusia*, Rauchenberger (1989) separated the 2 species from closely related species based on morphological differences of the intromittent organ of the males but separated *G. affinis* from *G. holbrooki* primarily on the structure of ventral gill arches (shape of the medial part of hypobranchial 1). In addition to the gonopodial characters used by Rauchenberger (1989) to distinguish among *Gambusia* species, male *G. holbrooki* also possess a series of prominent teeth on that are lacking in *G. affinis* (Page and Burr 1991; Fig. 2). Gonopodial characters are limited to males (which can be rare in population surveys, Walters and Freeman 2000) and ventral gill arch morphology is discernable by post-mortem dissection, but both characters are not easily used in the field, especially when species-categorization of the more abundant females is required.

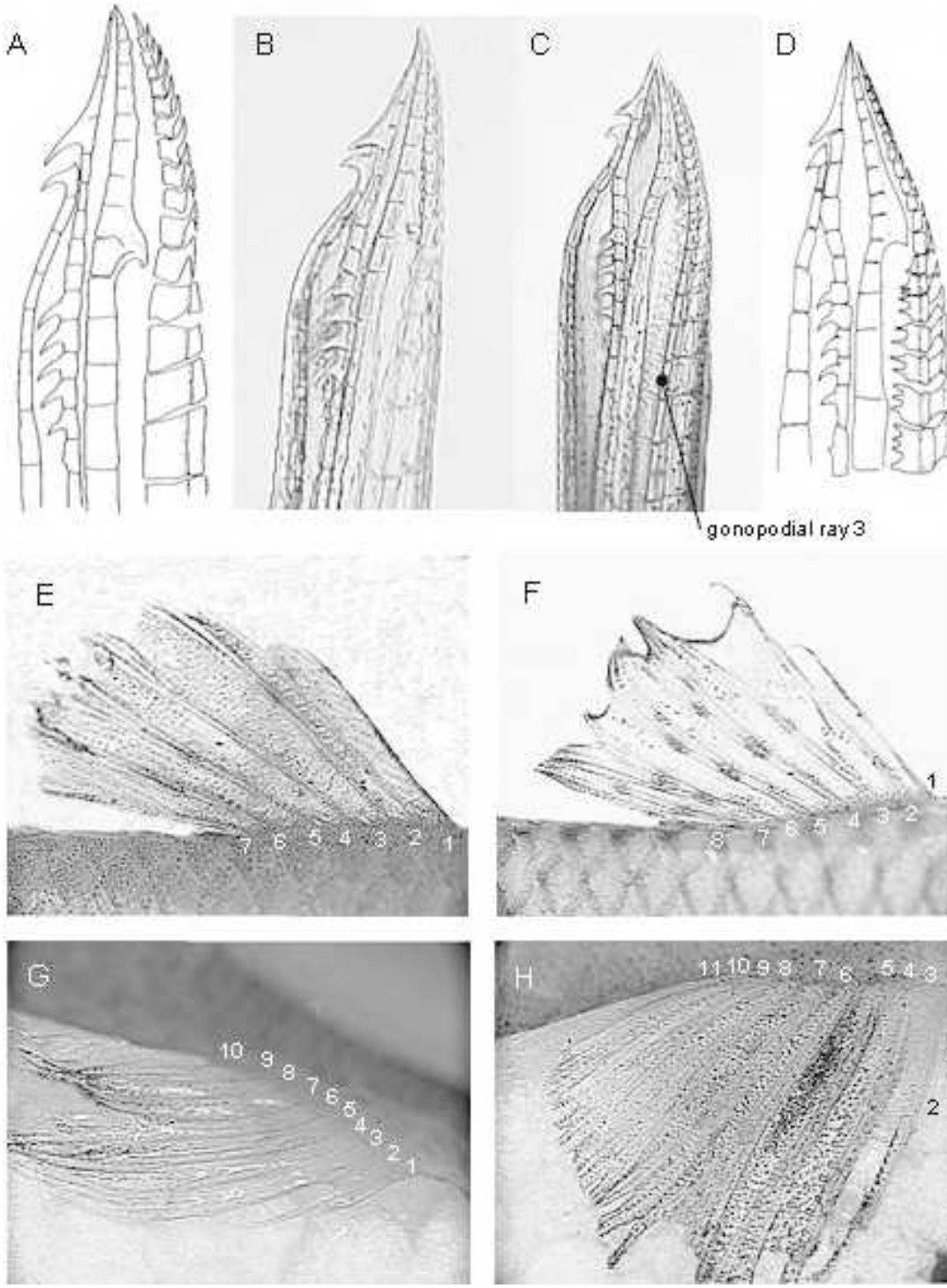

Fig. 2. Gonopodium, dorsal fin and anal fin of *Gambusia affinis* (gonopodium: A, B; dorsal fin: E; anal fin: G) and of *G. holbrooki* (gonopodium: C, D; dorsal fin: F; anal fin: H). Line drawings of the gonopodia (A, D) are from Rauchenberger (1989). Fin rays are numbered in panels E, F, G and H.

Many investigators have used the differences in fin ray counts of the females to distinguish between *G. affinis* and *G. holbrooki*. Walters and Freeman (2000) provide a succinct comparison of the discordant fin ray counts in the scientific literature. To reconcile differences in fin ray counts caused by previous investigators who differentially included or excluded split rays and rudiments, Walters and Freeman (2000) recommended counting all fin rays separately. Walters and Freeman (2000) separated the 2 species using dorsal and anal fin ray counts: *G. affinis* has 7 dorsal and 10 anal rays; *G. holbrooki* has 8 dorsal and 11 anal rays (Fig. 2). Walters and Freeman's (2000) counting method is not universally accepted; regardless, for a particular counting method for fin rays, *G. holbrooki* has one more fin ray in the dorsal and anal fins than does *G. affinis* as well as small serrations on the 3rd ray of the male anal fin (Moyle 2002).

The native geographic ranges of these 2 mosquitofish overlap in Alabama and are larger than those of other North American species that have comparatively restricted distributions in rivers and spring systems in the south-central United States and Mexico (Rauchenberger 1989, Page and Burr 1991). Sympatric populations of the 2 species have hybridized in western Alabama (Wooten et al. 1988, Scribner 1993) and can be found elsewhere in the southeastern United States (Walters and Freeman 2000); but the hybrid zones are hypothesized to be unstable (Walters and Freeman 2000). Interspecific hybrids between *G. affinis* and *G. holbrooki* often exhibit gonopodia of intermediate morphology (Angus and Howell 1996) and have the dorsal ray count of 1 species and the anal fin count of the other species (Walters and Freeman 2000). Even though hybrid individuals usually possess a total number of rays summed over the anal and dorsal fins that is intermediate to the total number of rays found in both species, natural variability in fin ray counts for both species can cause misidentifications (Angus and Howell 1996).

The instability of hybrid zones is caused by the differences in competitive abilities of the 2 mosquitofish species. *Gambusia holbrooki* is thought to outcompete and replace *G. affinis* in regions where the 2 species are sympatric. Scribner and Avise (1994) found that *G. holbrooki* was competitively superior to *G. affinis* in cage studies. Studies in an experimental stream suggested that *G. affinis* has greater dispersal tendency than does *G. holbrooki* (Rehage and Sih 2004). Locales where both species coexist are thought to represent recent introductions of *G. holbrooki* (Angus and Howell 1996, Walters and Freeman 2000).

The success of the 2 *Gambusia* species in a variety of habitats is due in part to life histories that favor rapid reproduction without the need to build nests, broad environmental tolerances, and the potential plasticity of phenotypic responses to environmental differences inherent in the genomes. The native habitats of mosquitofish are shallow, calm, productive temperate and tropical lowland lakes, ponds and streams (Meffe and Snelson 1989). Mosquitofish are often associated with submerged vegetation and found near to the shore (Hubbs 1971, Arthington et al. 1986). In streams and rivers, mosquitofish are usually found in calm backwaters and pools. Despite the fact that the majority of introductions outside the native range of mosquitofish are thought to be derived from relatively few populations, the heterozygosity (Robbins et al. 1987) and genetic differentiation (Hernandez-Martich and Smith 1990) among mosquitofish populations, especially *G. holbrooki*, in the native range are among the largest of fish species studied to date.

Like other members of the family Poeciliidae such as guppies (*Poecilia reticulata* (Peters)), mollies (*Poecilia* spp.) and swordtails and platies (*Xiphophorus* spp.), *Gambusia* spp. are livebearers with internal fertilization of the eggs and, following internal gestation of the eggs, produce free-swimming young. Females can store sperm. The number of fry produced in a brood ranges from 1 to about 300. Mean brood size of populations in nature ranges between 13 and 64 young (50: Krumholz 1948; 13–64: Gerberich and Laird 1985; 17.5–29.1: Reznick and Miles 1989); however, reproduction declines markedly during the late summer and early autumn (Sawara 1974, Reznick and Braum 1987, Daniels and Felley 1992, Vargas and Sostoa 1996). Neonate size is variable, ranging between 5–10 mm (0.2–0.4 in.; Swanson et al. 1996) and 0.7–1.5 mg dry weight (Reznick and Miles 1989); the sexes are indistinguishable. The mean number of fry surviving to 14–16 mm length is 5.4 individuals and survival rate is high after fry reach 16 mm length (Gerberich and Laird 1985). Young grow rapidly and become sexually mature in 3–10 wk at between about 11–15 mm standard length (SL) for males and 20 and 25 mm SL for overwintering females (Daniels and Felley 1992, Haynes and Cashner 1995). Both sexes are capable of maturing at comparatively smaller sizes ($\sim$ 13 mm) in elevated thermal environments (Specziar 2004). Young-of-year females derived from generations produced during the spring and early summer typically mature at smaller sizes (16–18 mm SL) than do overwintering females (Haynes and Cashner 1995). Individual females usually produce several broods (2–7) per season, the gestation period varies between 21 and 35 days depending on environmental conditions (Vargas and Sostoa 1996), and interbrood intervals range from 2.5–4 wk at 25°C (Reznick and Miles 1989, Haynes and Cashner 1995, Swanson et al. 1996). In nature, few

individuals survive longer than 15 months (Krumholz 1948, Fernández-Delgado and Rossomanno 1997, Cabral et al. 1999) and the age at death typically ranges from 30–70 wk after birth (Swanson et al. 1996). Vargas and Sostoa (1996) found that a very small proportion (0.5% with 2 annuli on dorso-lateral scales) of the females survived into 3 years. For most localities, reproduction is seasonal and individuals that mature late in the season usually overwinter.

Adult sex ratios frequently vary seasonally yet wild mosquitofish populations are often dominated by females (Britton and Moser 1982, Snelson 1989, Haynes and Cashner 1995, Zulian et al. 1995, Vargas and Sostoa 1996, Cabral and Marques 1999). Annual fluctuations in adult sex ratios are caused by differential maturation rates of the sexes and differential sex-specific mortality. Males mature sooner than do females (Hubbs 1971, Hughes 1985). Females are more susceptible than are males to piscivorous wading birds (Britton and Moser 1982) whereas males have shorter life spans than do females (Krumholz 1948) and perish more quickly when exposed to abiotic (temperature, hypoxia and other environmental stressors: Cech et al. 1985, Snelson 1989) and biotic stressors (overcrowding, starvation: Krumholz 1948). Gerberich and Laird (1985) stated that published reports suggested that a sex ratio of 1:5 (male:female) is optimal for sexual activity.

The fertility, offspring size, size at maturation, and length and synchrony of the reproductive season of mosquitofish vary with latitude and can affect the efficacy of biological control programs using *Gambusia*. Haynes and Cashner (1995) summarized the geographic trends in life histories of *G. affinis* in the United States and the reader is referred to this publication for an in-depth treatment of the topic and the associated literature. Briefly, Haynes and Cashner (1995) identified 5 latitudinal trends in *G. affinis* life histories: (1) fertility (brood size) decreases and offspring size increases from north to south; (2) large overwintered females in northern areas have a comparatively longer reproductive lifespan and produce more broods within a season than do females in southern populations and the reverse is true for young-of-year females that reproduce from spring until late summer/autumn; (3) the minimum size at 1st reproduction follows a seasonal pattern within populations (overwintering adults are larger than adults from young-of-year cohorts), but size at maturity tends to be smaller in southern and larger in northern and Hawaiian populations; (4) elevated winter mortality in northern populations (>99% in some populations) synchronizes reproduction early in the season, but high overwintering mortality is rare in southern populations and reproduction is not highly synchronized among different sized individuals during the early spring; and (5) mosquitofish populations reproduce year-round in Hawaii and cease reproduction during winter within the contiguous United States. However, mosquitofish in habitats receiving thermal effluent reproduced year-round in the southeastern United States and the percentage of reproductive females was directly related to water temperature (Meffe and Snelson 1989). The negative association between offspring number and size, length of the reproductive season, and vastly different levels of overwintering mortality across latitude will affect the potential for mosquitofish populations to increase after stocking for mosquito control and the persistence of mosquitofish populations in perennially problematic mosquito developmental sites.

Habitat stability also influences life history traits of mosquitofish populations. Genetic analyses revealed that water fluctuation in reservoir habitats was positively correlated with fecundity and negatively correlated with size at maturity (Stearns 1983a, 1983b). Environmental factors such as food levels have a dominant effect on *G. affinis* body size and most of the variation in life history traits of non-superfetating (carry 1 brood at a time) poeciliid species can be attributed to body size (Reznick and Miles 1989). High food, stable environments (such as highly controlled culture systems) would tend to favor genetically large females. If the relationships between body size and other life history characters determined for Hawaiian *Gambusia* populations by Stearns (1983b) hold for populations from other geographic regions, then large-bodied cultured fish should have comparatively high fecundity, high reproductive allotment (i.e., percentage of total dry weight invested in developing embryos) and small offspring. These traits would be expected to enhance the potential for increasing population size after stocking. Stressful or contaminated habitats may favor fish with small body size (*G. holbrooki*; Meffe 1992, Benton et al. 1994).

Meisch (1985) characterized the mosquitofish as an adventitious feeder and recent studies (Walters and Legner 1980, Bence and Murdoch 1982, Bence and Murdoch 1986, Morton et al. 1988, Lancaster and Drenner 1990, Cabral et al. 1998) confirm that *Gambusia* is an opportunistic omnivore. Even though mosquitofish concentrate feeding at the water surface, nektonic and benthic prey are taken readily. Diet composition is broad and includes small autotrophs (algae, diatoms, etc.), nematodes, microcrustaceans, aquatic insects, terrestrial insects, gastropods, and vertebrates including their own young (Swanson et al. 1996). Based on analyses of gut contents, between 6 and 13% of individuals in large populations are cannibalistic in nature (Walters and Legner 1980, Nesbit and Meffe 1993, Cabral

et al. 1998), although cannibalism rates can be much lower in some populations (~1%: Specziar 2004).

Feeding rate is strongly linked to growth rate (Swanson et al. 1996) and, like metabolism and activity, is influenced strongly by water temperature (Wurtsbaugh and Cech 1983, Moyle and Cech 2004). Feeding rate also is affected by fish size (Cech et al. 1980), sex (Hess and Tarzwell 1942), prey abundance (Vondracek et al. 1988), environmental factors such as dissolved oxygen concentration and salinity (Shakuntala and Reddy 1979, Cech et al. 1985), as well as other factors such as physical structure in the environment (Linden and Cech 1990), water velocity (Reddy and Pandian 1974) and time of day (Moyle and Cech 2004). Even though the common name is the mosquitofish, *Gambusia* spp. are not monophagous and cannot survive and grow on a diet of only immature mosquitoes (Reddy and Pandian 1972, Gerbeich and Laird 1985).

Mosquitofish population size is dependent on many factors such as food availability and mortality from predation and disease (Wainwright et al. 1984, Swanson et al. 1996). Mosquitofish population growth following stocking is typically asymptotic (Farley and Younce 1979, Stewart and Miura 1985). Miura and Takahashi (1987) suggested that population growth follows a logistic curve where rapid population growth after stocking eventually slows and then stabilizes around the carrying capacity of the environment. The large fluctuations in mosquitofish trap catches observed in some rice fields (Wainwright et al. 1984), however, suggest populations do not closely track the carrying capacity of environments. Large fluctuations in mosquitofish population size increase the likelihood of control failure during population crashes and of potentially detrimental effects on the ecosystem from overconsumption during population peaks. Swanson et al. (1996) point out that reproduction is often synchronized in mosquitofish populations by temperature and photoperiod cues; however, handling stress and/or environmental stress can significantly affect reproduction for transplanted populations. Mortality caused by cannibalism, overexploitation of food resources, disease or predation (Swanson et al. 1996) after the 1st bout of reproduction, when mosquitofish populations are either comparatively stable or declining, can be catastrophic (Wainwright et al. 1984, Botsford et al. 1987). Moreover, sustained mosquito control in perennially problematic habitats (e.g., man-made wetlands receiving nutrient-rich wastewater) can be compromised by seasonal declines in reproduction during late summer and by natural overwintering mortality that reduce mosquitofish population size during the following spring and early summer when populations of *Culex* reach annual maxima (Walton 2002).

An unintended consequence of the widespread use of mosquitofish as a biological control agent for mosquitoes has been the movement of mosquitofish into habitats where mosquito control is not required. For example, field observations suggest that *Gambusia* colonization of seasonally disjunct or restricted habitats containing depauperate faunas has been detrimental to some fish species in the southwestern United States (Courtenay et al. 1986, Courtenay and Meffe 1989, Courtenay and Williams 1992). Mosquitofish actively disperse as population densities and competition for food and space increase. Robbins et al. (1987) found that three-quarters of dispersing fish were females, nearly half of the individuals were close to parturition (i.e., contained eyed embryos) and > 50% of the broods were from multiple paternity. Large (> 22 mm SL) female *G. holbrooki* preferentially dispersed downstream and females dispersed more readily than did male and juvenile fish at low (9 mm/sec) and high (109 mm/sec) flow rates (Congdon 1994). Mosquitofish also are prone to wash out from habitats by erosive floods (Courtenay and Meffe 1989). Ward et al. (2003) found that the mean failure velocity, the water velocity at which fish could no longer maintain position in the water column, of *G. affinis* (37.5–44.9 mm TL) was 38.5 cm/sec. Because female mosquitofish can store sperm, 1 inseminated individual is sufficient to found a new population.

In contrast to insect parasitoids that are often closely linked to a particular pest species and typically have little or no impact on non-target organisms, control of a pest organism (i.e., mosquitoes, algae and rooted aquatic plants) by fish cannot be achieved without impacts on non-target species (Courtenay and Williams 1992). The life histories and wide environmental tolerances of mosquitofish make them ideal for biological control in environments unsuitable for many other fish species; yet, these same characteristics make mosquitofish an invasive species.

PRODUCTION

One subject that has changed considerably since the previous version of this text (Chapman 1985) is the development and prevalence of contained culture systems for mosquitofish. Demands for mosquitofish by mosquito control agencies often exceed the capacity of sources in nature to produce mosquitofish (Hoy 1985, Meisch 1985). Whereas in most instances the agricultural, domestic and industrial water sources containing *Gambusia* do not strictly qualify as "native" collections, the practicality and predictability of fish collection from these

sources are usually much lower than is required by mosquito control agencies that utilize mosquitofish in large-scale biological control programs. For example, the total annual use of mosquitofish in California, 6,560–6,750 kg *Gambusia*/year, was met by a majority (58% of 50 districts that responded to a survey on fish use) of Mosquito/ Vector Control Districts (MVCDs) by culturing or harvesting fish; 38% of MVCDs purchased approximately 589 kg of mosquitofish from other districts or a commercial aquaculture company (< 9% of biomass used annually; Scott 2006). Demand for mosquitofish in rice fields alone (assuming a minimum stocking rate of ~ 0.22 kg mosquitofish/ ha) exceeds the maximum supply potential of California MVCDs (9,804 kg *Gambusia*/year) by 4.7 times (Scott 2006). Culture systems designed for cost-effective production of fish are increasingly part of modern mosquito control operations. The type of system appropriate for a mosquito control agency will be determined by the needs and resources of that agency. This chapter provides an overview of the subject; the reader is again referred to Swanson et al. (1996) for cogent discussion of culture systems and the environmental and biological conditions for mosquitofish rearing.

Currently, mosquitofish culture is carried out principally using 4 methods: commercial aquaculture operations, pond culture in outdoor earthen ponds, controlled-culture conditions (usually indoors), and in cages within mosquitofish source habitats. Even though fish are reared in earthern ponds in both commercial aquaculture operations and outdoor culture systems, a distinction is made here between commercial aquaculture operations that customarily include regimented supplemental feeding, and pond culture systems where supplemental feed is not added routinely after stocking fish. The per capita cost of mosquitofish to vector control programs is likely to differ between the systems. Current prices for mosquitofish from commercial aquaculture operations can be as low as $0.10 (U.S.) per fish (S. Su, Coachella Valley Mosquito and Vector Control District, Indio, CA; personal communication) but may be higher. The per capita cost of fish reared under controlled-culture conditions will be a function of the number of fish reared versus the capital investment in the culture system and building, and the operational costs including personnel, utilities, feed, antibiotics, etc. Current costs range between $0.05–0.10 per fish for high volume culture systems including indoor and outdoor rearing facilities to $0.10–0.25 per fish for indoor rearing facilities (S. Su, Coachella Valley Mosquito and Vector Control District, Indio, CA; personal communication). The current per capita cost of *Gambusia* is not appreciably different from the cost per fish in 1985 which ranged between $0.08 and $0.22 (Reynolds 1977, Meisch 1985); although, after adjustment for inflation the current per capita costs in 2004 would be about 65% lower that than in the mid 1980s.

Outdoor Rearing Facilities: Commercial Aquaculture and Earthen Ponds

Meisch (1985) discussed taking advantage of concurrent production of mosquitofish and species such as minnows and catfish under aquaculture. Haq et al. (1991) recommended the culture of *Gambusia* along with edible fish in village ponds in malarious regions of India. *Gambusia* is viewed as an undesirable pest species in many commercial aquaculture operations; considerable mosquitofish biomass produced as a by-product of aquaculture operations is wasted when the commercial fish species are harvested. Mosquitofish are often subject to substantial mortality by components of culture systems (e.g., water agitators) and harvesting methods (e.g., temperature shock). Meisch (1985) recommended that studies of handling, transportation, storage methods and temperature tolerances for mosquitofish be carried out to evaluate the potential for commercial aquaculture as a source of stock for mosquito control. To date, few commercial operators want to focus on rearing *Gambusia* and consequently commercial aquaculture operations are not a primary source of mosquitofish for mosquito abatement and vector control districts.

Pond culture systems use outdoor impoundments and do not utilize the strict control of environmental factors and food supply applied to contained culture systems. Pond culture systems are often enriched with fertilizers and stocked with natural assemblages of zooplankton before adding fish (Schon 1997). Problems/factors that reduce fish production and make harvesting fish difficult in pond culture systems include contamination/colonization by predators and competitors of mosquitofish, overexploitation of food resources, parasites and diseases, suboptimal physicochemical conditions for survival and growth (e.g., cold water temperatures) and the development of emergent and/or submerged vegetation (Meisch 1985, Downs et al. 1986). Multiple harvests of mosquitofish, supplemental feeding and polyculture usually increase mosquitofish production (Newton et al. 1977, Robison et al. 1983, Meisch 1985). Overwintering mortality of mosquitofish from piscivorous birds in outdoor rearing ponds can be high (60–99%) and is significantly larger than for contained systems where overwintering mortality is < 8–10% (Coykendall 1977, Schon 1997). Covering ponds with netting can help to reduce losses to birds (Coykendall 1977, Meisch 1985). As compared to contained culture systems, pond culture systems require larger amounts of land, often need piscivore control and, depending on pond configuration, can be more difficult to harvest

(Swanson et al. 1996). An advantage of pond culture systems to contained culture systems is the comparatively low cost of mosquitofish production.

Mosquitofish production will be a function of food supply and other environmental factors. Ponds receiving heated discharge from a milk processing plant in Glenn County, CA produced 500–850 kg *Gambusia* per ha (446–758 lb per acre), did not require supplemental food, but did require frequent harvesting and aeration to reduce mortality from hypoxia (Swanson et al. 1996). Ponds in fish polyculture in Arkansas yielded approximately 91 kg *G. affinis* per 0.4 ha, which was about 34% of the total fish biomass (Newton et al. 1977); total fish biomass yield, 669 kg per ha, was within the range of the highly enriched California ponds. However, without supplemental feed for the species in the polyculture, outdoor pond systems produced only 56.8 kg mosquitofish per ha (50.7 lb per acre). Annual production (ash-free dry weight: AFDW) of *G. holbrooki* in rice fields in Portugal was 3.101 g per m^2 per year; mean biomass (AFDW) was 2.896 g per m^2 (Cabral and Marques 1999).

Controlled-Culture Systems

The application of contained culture systems for mosquitofish was in its infancy in 1985 (Hoy 1985) and experience with mosquitofish rearing in these systems has advanced considerably during the last 20 years. Hoy (1985) concluded that fish production by closed-system rearing facilities could not be cost competitive with chemical control methods for mosquito control in rice fields unless carrying capacity of the culture system or mosquitofish growth rates could be increased above those in his study. Controlled culture systems use tanks or raceways that are usually enclosed within buildings (Swanson et al. 1996). These systems typically focus on optimizing survival and production in 2 components of a mosquitofish production system: brood stock and offspring (Downs et al. 1986). Environmental factors (e.g., temperature) can be controlled and manipulated (e.g., dissolved oxygen, photoperiod, flow rate) to enhance mosquitofish production. Population characteristics also are amenable to manipulation to optimize mosquitofish production in contained culture systems. For example, biomass gain and fry production were enhanced by manipulating fish density (1 individual per liter) and sex ratio (1:1 males to females), respectively (Downs and Beesley 1983, Downs et al. 1986). Hoy (1985) estimated that growth rate of *G. affinis* in nature is about 11% per day which is about half that achievable in captivity (>20% per day: Wurtsbaugh and Cech 1983, Hoy 1985). Such daily growth rates greatly exceed those reported in Gerberich and Laird (1985) for small earthern ponds (1% per day; food conversion ratio of 1.88). Unlike the pond culture systems that are typically closed systems where water is added to replace that lost to evaporation, contained culture systems use flow-through or recirculating water supplies. The food supply is strictly controlled and these systems require monitoring and control of water quality (Swanson et al. 1996) as well as personnel highly trained for fish and system maintenance.

The maintenance and power requirements (e.g., filters, pumps, cleaning of holding tanks/runways), cost of the water supply and capital investment make contained culture systems a comparatively expensive mosquitofish rearing approach. A 12-fold volume exchange per day (four 2,270-liter runways with total flow at 76 liters/min = 109,008 liters/day) is used for overwintering fish maintained in a flow-through system by the Sacramento-Yolo MVCD. A recirculating tank-based system required only 3–5% volume addition per day to replace water lost to evaporation (Contra Costa MVCD; Swanson et al. 1996). After filling the system (4 tanks at 37,850 liters), daily water use was < 2% that used in the flow-through system. Despite the comparatively high cost of these systems, contained culture systems provide a viable approach for (1) MVCDs that have a high demand for larvivorous fishes and (2) urban and suburban MVCDs that do not have the local sources (commercial as well as native) of larvivorous fish and do not have the land/desire for large rearing ponds.

Advantages of contained culture systems include improved overwintering survival and an accessible, healthy supply of mosquitofish for stocking mosquito-production sites early in the season (Swanson et al. 1996). Fish in contained culture systems are typically treated with paracides and antibiotics, and reared in high water quality (e.g., water that had undergone carbon filtration). Approximately 93% of 127 kg (279 lb) stocked in the autumn was collected for stocking in April compared to 37% of stocked mosquitofish biomass in outdoor ponds (Schon 1997). Moreover, mosquitofish populations in ponds stocked with fish that had been overwintered indoors produced more fish than did *G. affinis* populations in ponds stocked with the fish that had been overwintered outdoors in earthern ponds (Schon 1997).

Cage Systems

Cage systems (Coykendall 1980) are used to confine fish, especially in situations where piscivorous fish are present or where habitat characteristics make harvesting difficult. The problems facing this approach are similar to those found in pond culture systems (Swanson et al. 1996) in addition to having a variety of drawbacks such as practical-sized cages for rearing fish being cumbersome, susceptibility to damage and restricted

production due to limitations on cage size (Coykendall et al. 1991, Abshier et al. 1991). Cage culture systems are hardly ever used for large-scale mosquitofish culture although cage-style traps can effectively capture large numbers of mosquitofish in some habitats. Staines (1970) reported as many as 100,000 fish were collected in a single day in wastewater ponds. Plans for a cage trap are provided by Sholdt et al. (1972).

Native Collections

Mosquitofish collections from native sources rarely provide sufficient numbers of fish to fulfill the needs of MVCDs that practice large-scale biological control such as those for irrigated agriculture. But local sources can provide an adequate number of fish for vector control programs that have small-scale programs such as providing *Gambusia* for stocking in ornamental ponds, swimming pools and other small mosquito developmental sites common in urban and suburban settings. Over one-half of 50 MVCDs in California that use *Gambusia* for mosquito control harvest fish from local sources (Scott 2006). Mosquito control programs within the native geographic ranges of the 2 *Gambusia* species may find a sufficient number of fish to meet their needs if demand for *Gambusia* is low, such as situations where large-scale agricultural operations such as rice farming are not present or when mosquito abatement strategies rely primarily on mosquito control approaches incompatible with fish use. Because of the potential negative impact of parasites and diseases (Ganzhorn et al. 1992, Courtenay and Williams 1992) on other species as well as native mosquitofish populations, wide-scale geographic and interwatershed transfers should be discouraged. If such transfers are required, then they should be carried out with care and in concert with applicable state, regional and federal regulations.

Handling stress and/or environmental stress can significantly affect the success of transplanted populations so care is needed when transporting and handling fish derived from native sources as well as culture operations. The American Fisheries Society, American Society of Ichthyologist and Herpetologists and American Institute of Fisheries Research Biologists recently published a guide of fish care that specifically addresses issues relating to laboratory care for fishes (AFS, ASIH, AIFRB 2004). The new guidelines have been developed to replace the ASIH, AFS, AIFRB (1988) guidelines and to expand the coverage to include laboratory research. Erickson (2003) provides a comprehensive review of information resources regarding fish welfare. Although these guidelines pertain to use of fish for research, they provide some commonsense rules of thumb applicable to the transport and handling fish for biological control programs.

Transport procedures must be designed to minimize the effects of stress and thereby reduce immediate and delayed losses. Do not remove the fish from water if possible and minimize exposure to air (Swanson et al. 1996). Transportation in aerated tanks or coolers is recommended. Transport containers should be in good condition and disinfected before use. A weak bleach solution (5% bleach) followed by multiple rinses with tap water can be used to disinfect transport containers. After rinsing, soaking the transport container overnight and discarding the soak water before use will assure that all remnants of the hypochlorite solution are removed.

The weight of fish that can be transported safely depends on efficiency of the aeration system, duration of the haul, water temperature, and fish size. Maintaining acceptable levels of dissolved oxygen, carbon dioxide, temperature, ammonia, and pH during transport is essential to promoting the survival of fish. Water replacement or battery-powered aeration systems should be used during transport. Water conditioner products that reduce the effects of ammonia, chloramines, chlorines and other potentially toxic materials, that buffer pH, and that reduce stress during transport/holding should be used. Sodium chloride (NaCl) and nitrofurozone (Furacin®) should be added to the water from the collection site at concentrations of 0.5% and 20 mg/liter (AI by weight), respectively, to minimize osmotic stress and external bacterial infections (Swanson et al. 1996). Fish should be transported in water from the collection site whenever possible. Aerated, de-chlorinated, conditioned tap water is an acceptable substitute.

Fish should be acclimated to a new environment by gradually replacing the water in transport units with water from the new environment. This is a commonly used practice to provide time for acclimation to new temperature and water quality conditions. Care should be taken to provide water as similar as possible to that of the collection site. Provided that temperature differentials are not >5°C (> 9–10°F), exchanging one half the volume of the transport water with water from the new environment and holding the fish for a minimum of 1 h before transfer to the new environment has provided nearly 100% survival of transferred mosquitofish. Longer periods of acclimation (24–48 h) and smaller proportional volume additions will be required if the water temperature or water quality of the new environment differs appreciably from the collection site.

TOXICOLOGICAL STUDIES

Chemical larvicides and adulticides remain important components of mosquito abatement programs in many regions of the United States,

particularly the Southeast (Tietze et al. 1991, FCCMC 1998). Mosquitofish are not stocked in Florida as extensively as is done in California (FCCMC 1998); however, larvivorous fish are an important biological control agent for mosquitoes. In other regions of the United States, such as central and southern California, where mosquitofish are stocked as components of integrated mosquito management (IMM) programs, vector control agencies have shifted away from using conventional chemical insecticides to using microbial larvicides and other approaches focused against immature mosquitoes to complement mosquito control by fishes. Nevertheless, adulticides are still used against mosquitoes during public health emergencies and in instances where the large areas requiring mosquito control preclude the use of more costly biologically based larvicides. The number of conventional chemical insecticides available for public health uses continues to decline as registrations for use are not renewed and the development of new insecticides has not kept pace with the number of chemicals that have been removed from approved use. Managing resistance in the target insect populations to approved insecticides and understanding the potential consequences of pesticide usage on beneficial organisms are critical for the development of sound IMM strategies.

Whereas comparatively few recent studies have focused on the mortality effects of insecticides on mosquitofish during the period since Meisch's (1985) review, an extensive literature on the effects of insecticides on *Gambusia* spp. and other fishes exists. Toxicological studies have focused on diverse subjects including accumulation of the compound of interest and its metabolic byproducts in the body, behavioral effects, physiological (enzyme, hormonal, biochemical) effects, morphological effects (growth and tissue studies), reproductive effects especially those resulting from chronic exposure, population and ecosystem studies. The majority of the toxicological findings now can be accessed electronically on the U.S. Environmental Protection Agency's *Ecotox Database* (http://www.epa.gov/ecotox/) and on the Pesticide Action Network's *PAN Pesticide Database* (http://www.pesticideinfo.org; Orme and Kegley 2004).

Table 1 summarizes the mortality effects of insecticides used for mosquito control and herbicides used primarily for weed control in rice fields on *G. affinis* and *G. holbrooki*. Meisch (1985) concluded that organochlorines (e.g., DDT, toxaphene) were the most toxic substances to fish, organophosphorus compounds (e.g., parathion, chlorpyrifos) were extremely toxic to invertebrates but less toxic to fish, and carbamates (e.g., carbaryl and carbofuran) were the least toxic insecticides to fish tested to date. Mittal et al. (1991) found that the relative toxicities to *G. affinis* of classes of pesticides were pyrethroids > organochlorine insecticides (DDT, gamma-HCH) > organophosphorus pesticides (malathion, fenthion, monocrotophos and temephos). Organochlorine insecticides are no longer used in the United States for mosquito control and are not listed in Table 1.

Among the chemical insecticides used currently for mosquito control, synthetic pyrethroids (i.e., permethrin, resmethrin) are acutely toxic to mosquitofish (Tietze et al. 1991, 1995; Lawler et al. 2003). Pyrethrum, the naturally occurring compound on which the synthetic compounds are based, is also highly toxic to mosquitofish (Fabacher and Chambers 1972). Pyrethroids are 100–1000 times more toxic to *Gambusia* spp. than are other insecticides routinely used for mosquito control (Table 1). Mittal et al. (1991) found that lambda-cyhalothrin was the most toxic to *G. affinis* among several synthetic pyrethroids (deltamethrin, cypermethrin and fenvalerate); however, mosquitocidal concentrations (0.0667–0.0954 μg/liter for 4th instar *Cx. quinquefasciatus* Say) had no effect on *G. affinis* (Mohsen et al. 1995). Pyrethroids are essentially nontoxic to mammals (Matsumura 1985).

Generalizations regarding the relative toxicity of particular classes of other conventional chemical insecticides to mosquitofish are difficult to make because the median mosquitofish mortality often differs appreciably among compounds within each class of insecticide (Table 1). Some of this variation in mortality is caused by differences in formulations, the size or age of fish tested and the period of exposure to the insecticides among studies. Because a rapid reproductive response by stocked mosquitofish is critical for successful biological control of mosquitoes and the susceptibility of fry and fingerlings to the insecticides is greater than for adult fish, mortality of young fish would seem a reasonable metric for basing recommendations for IMM programs that either combine insecticide usage with mosquitofish or are potentially affected by insecticide applications (i.e., drift). Chronic effects from long-term exposure to low levels of pesticides also might be a concern (Wickramasinghe and Costa 1986). The nearly 12-fold difference in 24-h LC_{50} lessened after 48 h of exposure to a 5-fold difference between the most vs. least toxic chemicals [LC_{50}: malathion = temephos = naled < fenthion < fenoxycarb: Tietze et al. 1991)]. Exposure to sublethal concentrations of malathion (0.01 and 0.02 mg/liter) caused detrimental histopathological effects in the gill tissue of *G. affinis* (Cengiz and Unlu 2003). Mosquitoes (*Anopheles quadrimaculatus* Say) were less susceptible to lambda-cyhalothrin than were predatory insects (Dennett et al. 2003) and a single application of lambda-cyhalothrin at

Table 1. Toxicity of various herbicides and insecticides to mosquitofish. Modified from Swanson et al. (1996).

Chemical	Exposure (hr)	LC_{50}, LD_{50} (mg/liter)	Mortality (%)	Mortality (mg/ liter)	Reference
Herbicides					
Aquashade (dye)	–	–	safe	–	Downs (1991)
bentazon	24	4,418			Leung et al. (1983)
bentazon	96	3,874			Leung et al. (1983)
bentazon	48	600			Sun (1987)
copper sulfate	24		89	7	Sjogren (1972)
copper sulfate	75	10+	–	–	Ahmed (1976)
Cutrine-plus	24	290	–	–	Leung et al. (1983)
Cutrine-plus	96	170	–	–	Leung et al. (1983)
diquat	24	723	–	–	Leung et al. (1983)
diquat	96	289	–	–	Leung et al. (1983)
diuron	72	10+	–	–	Ahmed (1976)
endothal	72	10+	–	–	Ahmed (1976)
2,4-D ester	24	7	–	–	Hansen et al. (1972)
glyphosate (41% SL)	48	6.2			Sun (1987)
glyphosate	–	–	safe	–	Downs (1991)
molinate	24	24.24	90	31.31	Davey et al. (1976)
molinate	48	18.42	90	24.98	Davey et al. (1976)
paraquat	24	1,080			Johnson (1978)
paraquat	48	751			Johnson (1978)
paraquat	96	604			Johnson (1978)
paraquat (24% EC)	48	5.1			Sun (1987)
propanil	24	11.21	90	20.58	Davey et al. (1976)
propanil	48	8.45	90	12.14	Davey et al. (1976)
simazine	–	–	safe	–	Darwazeh and Mulla (1974)
thiobencarb (50%EC)	48	0.5			Sun (1987)
thiobencarb (Saturn)	48	1.165			Sun (1987)
thiobencarb (static: EC)	96	3			Schaefer et al. (1983)
thiobencarb (flow-through: EC)	96	1.3			Schaefer et al. (1983)
thiobencarb	96	1.5			Persichino et al. (1998)
Insecticides					
Carbamates					
carbaryl		1.45			Areekul (1986)
carbaryl (80% WP)	24	40.0			Chaiyarach et al. (1975)
carbaryl (80% WP)	48	35.0			Chaiyarach et al. (1975)
carbaryl (80% WP)	72	32.4			Chaiyarach et al. (1975)
carbaryl (80% WP)	96	31.8			Chaiyarach et al. (1975)
carbaryl	96	1.40			Carter and Graves (1972)
carbaryl	24	1.30			Krieger and Lee (1973)
carbaryl (Sevin)	48	0.736			Li and Chen (1981)
carbofuran	96	0.3			Carter and Graves (1972)
carbofuran	48	1.5			Sun (1987)
carbofuran	24	0.96	90	2.84	Davey et al. (1976)
carbofuran	48	0.57	90	1.20	Davey et al. (1976)
fenoxycarb	72	0.07			Mohsen et al. (1989)
fenoxycarb	24	1.05	–	–	Tietze et al. (1991)
Chitin-synthesis inhibitors					
diflubenzuron	96 fry	100	0		Miura et al. (1975)
Insect growth regulators (IGRs)					
methoprene	288	–	0	200	Ellgaard et al. (1979)
methoprene	24	–	0	3×10^4	Tietze et al. (1991)
Microbial larvicides					
Bacillus sphaericus	24	–	0	520	Mulligan et al. (1978)
B. thuringiensis subsp. *israelensis*	336	–	0	1×10^7	Garcia et al. (1980)
B.t.i.	24	–	0	1×10^4	Tietze et al. (1991)
Organophophates					
chlorpyrifos	24	4	–	–	Hansen et al. (1972)
chlorpyrifos	24	1.4	90	7.27	Davey et al. (1976)
chlorpyrifos	48	0.44	90	2.1	Davey et al. (1976)
chlorpyrifos	72	0.26			Davey et al. (1976)

Table 1. Continued.

Chemical	Exposure (hr)	LC_{50}, LD_{50} (mg/liter)	Mortality (%)	Mortality (mg/ liter)	Reference
chlorpyrifos	96	0.28			Carter and Graves (1972)
chlorpyrifos	36	0.215–0.23			Ferguson et al. (1966)
chlorpyrifos	24	0.19–0.2			Ahmed and Washino (1976)
diazinon (Basudin 93% PU)	48	1.273			Li and Chen (1981)
diazinon	NR	12.800			Areekul (1986)
fenthion	24	2.94	–	–	Tietze et al. (1991)
fenthion	24	0.1			Johnson (1977)
fenthion	48	1.00			Darwazeh and Mulla (1974)
malathion	96	0.3			Cano et al. (1999)
malathion	96	0.7			Li and Fan (1996)
malathion	48	1.061			Culley and Ferguson (1969)
malathion	24	0.15			Mohsen et al. (1989)
malathion	96	0.2			Naqvi and Hawkins (1988)
malathion	24	12.7	–	–	Tietze et al. (1991)
malathion	48	3.44	–	–	Tietze et al. (1991)
malathion	24	–	40	0.05	Lewallen (1959)
malathion	24	2	–	–	Hansen et al. (1972)
methyl parathion	72	–	0	6	Ahmed et al. (1970)
malathion (Cythion)	48	5			Premkishore and Chandran (1988)
naled	24	3.5	–	–	Tietze et al. (1991)
naled	24	–	3	0.3	Lewallen (1959)
parathion	24	–	33	0.004	Lewallen (1959)
parathion	24	–	0	0.05	Ahmed et al. (1970)
parathion	24	–	100	0.3	Ahmed et al. (1970)
parathion	24	0.2–2.0			Kynard (1974)
parathion	24	0.64	90	3.71	Davey et al. (1976)
parathion	48	0.26	90	1.43	Davey et al. (1976)
parathion	48	0.32			Mayer and Ellersieck (1986)
parathion	48	0.35			USEPA (2000)
parathion	24	0.085–0.2			Ahmed and Washino (1976)
parathion	48	0.048			Culley and Fergusen (1969)
parathion	48	0.35–0.95			Chambers and Yarbrough (1974)
temephos	24	5.6			Tietze et al. (1991)
temephos	24	0.003			Mohsen et al. (1989)
Pyrethroids					
deltamethrin	24	1.0			Mulla et al. (1978)
deltamethrin	48	1.0			Mulla et al. (1978)
deltamethrin	24	0.83			Mohsen et al. (1989)
deltamethrin	48	1.80			Sun (1987)
deltamethrin	48	0.5–1.0			Thvbaud (1990)
deltamethrin	24	1.0–2.0			Thvbaud (1990)
lambda-cyhalothrin	adults		100	5.8 g AI/ha	Lawler et al. (2003)
lambda-cyhalothrin		0.0022			Mittal et al. (1991)
permethrin	96	0.015			Jolly et al. (1978b)
permethrin	96	0.015			Jolly et al. (1978a)
permethrin	24	0.100			Mulla et al. (1978)
permethrin	48	0.097			Mulla et al. (1978)
permethrin (2–5 d)	24	0.00604			Tietze et al. (1995)
permethrin	48	0.00429			Tietze et al. (1995)
pyrethrum	24	0.027			Fabacher and Chambers (1972)
resmethrin	24	0.00693			Tietze et al. (1991)
resmethrin	48	0.00524			Tietze et al. (1991)
Surface insecticides: oils & monomolecular films					
GB-1111	24	593			Tietze et al. (1991)
GB-1111	48	372	–	–	Tietze et al. (1991)
Genapol OXD-080	96	2.9			Cabral et al. (1999)
Genapol OX-080	96	0.12–0.22			Cano et al. (1999)
Genapol OX-080	A96	0.0008%			Cabral et al. (1997)
Genapol OX-080 (+ Solvenon at 20%)	A96	0.0006%			Cabral et al. (1997)

the label rate for control of agricultural pest insects in rice caused 100% mortality of *G. affinis* in rice fields (Lawler et al. 2003). However, 24-h laboratory exposure at lambda-cyhalothrin concentrations (0.0667–0.0954 μg/liter) sufficient to cause 50–90% mortality in 4th instar *Cx. quinquefasciatus* Say had no harmful effect on *G. affinis*. Even though mortality of free-ranging fish in nature is often lower than that for fish in laboratory toxicological studies (Meisch 1985) because some pesticides absorb quickly to soil and plant material (Maund et al. 1998) and fish avoid areas containing irritants (Hansen et al. 1972), extreme care is needed when applying conventional chemical insecticides as part of integrated control programs.

Consideration of the impacts of conventional chemical insecticides on beneficial organisms transcends the consideration of the impacts on mosquitofish alone. For example, the toxicity of other mosquito control agents such as diflubenzuron to juvenile *G. affinis* is comparatively less than the carbamates, organophosphorus compounds and pyrethroids (Table 1) but this chemical is extremely toxic to aquatic invertebrates (Miura et al. 1975). Special use permits for this larvicide are required in regions of the United States where use is still permitted and the chemical cannot be applied to natural systems (FCCMC 1998). Many of the prey of mosquitofish are more sensitive than are mosquitofish to the effects of pesticides (see listings in Orme and Kegley 2004). Most mosquito adulticides used in the United States have very low application rates and are applied under conditions that reduce the likelihood of unintended detrimental consequences. Therefore, chemical mosquito control agents have good safety margins against drift. Purposeful application or unintentional drift of insecticides for agricultural pest control can have detrimental effects on the crustacean and insect food resources critical for mosquitofish reproduction and for other organisms in aquatic ecosystem food webs.

The new generation of larvicides such as the insect growth regulator methoprene and bacterial larvicides (*Bacillus thuringiensis* subsp. *israelensis* de Barjac and *B. sphaericus* Neide) are among the safest mosquito control agents for fish (Table 1). These mosquito control agents have the additional benefit of being nontoxic to non-target organisms when applied at mosquitocidal rates (Walton and Mulla 1992, Lawler et al. 2000). The use of bacterial larvicides in IMM programs provides an increased level of safety for fish and non-target organisms to that of conventional chemical larvicides such as temephos (Kramer et al. 1987, 1988; Mittal et al. 1991, FCCMC 1998; Walton and Mulla 1989; Walton et al. 1991).

Surface insecticides such as mosquitocidal oils (Golden Bear: GB-1111®) and monomolecular films (e.g., isostearyl alcohols: Arosurf MSF®, Agnique MMF 5996®) are safe for mosquitofish when applied at mosquitocidal concentrations (Tietze et al. 1991, FCCMC 1998; Table 1). The latter products may be used safely in potable waters. However, not all surface agents are recommended for use in habitats containing mosquitofish. The non-ionic surfactant, Genapol OXD-080®, is a fatty alcohol polyglycol ether used at 50 mg/liter for crayfish control in rice fields in Central Portugal (Cabral et al. 1999). Based on population models that incorporated potential sublethal effects of surfactant application on *G. holbrooki*, Cabral et al. (2001) suggested that low-level contamination circa 0.75 mg/liter in mosquitofish refuges such as irrigation canals would cause significant damage to mosquitofish populations.

Herbicides and algacides may be routinely used in habitats containing mosquitofish or in habitats that are connected to mosquitofish habitats. Herbicides are an important management tool for weed control in rice fields that might contain *Gambusia*. For example, 18 herbicides are considered for application to rice fields in Arkansas (Scott 2004). Mosquitofish are more tolerant to herbicides than insecticides (Meisch 1985) but herbicides are usually applied at concentrations much higher than insecticides (Jones 1964). The LC_{50} of several herbicides (benthazon, paraquat, thiobencarb) for rice culture to mosquitofish are orders of magnitude higher than for insecticides (Table 1). Although the toxicities of other herbicides potentially used in rice growing have not been determined for mosquitofish, herbicide-induced mortality has been studied for fish species such as the bluegill sunfish (*Lepomis macrochirus* R.) and the sheepshead minnow (*Cyprinodon variegates* Lacepède). The toxicities of these herbicides fall into 4 categories: (1) not acutely toxic to slightly toxic with 96-h $LC_{50} >$ 100,000 μg/liter: sulfonylurea herbicides (e.g., thifensulfuron-methyl, tribenuron, bensulfuron, halosulfuron) and imazethapyr; (2) slightly toxic with 96-h LC_{50}s 32,000–62,000 μg $liter^{-1}$: quinclorac (Facet®), clomazone, and diphenyl ethers (acrifluorfen); (3) moderately toxic with 96-h LC_{50} ~2,000 μg/liter: carfentrazone-ethyl; and (4) moderately to highly toxic with mean 96-h $LC_{50} < 1{,}000$ μg/ liter: pendimethalin (96-h LC_{50}: 199–1,040 μg/liter), fenoxaprop (96-h LC_{50}: 310–2,860 μg/liter).

FIELD APPLICATION

Swanson et al. (1996) recommend that safe and effective use of mosquitofish requires (1) a working knowledge of operational techniques, (2) a thorough evaluation of the stocking sites, (3) use of appropriate stocking methods, (4) regular monitoring of stocked populations, and (5) an

Fig. 3. Ardeids in duck hunting club ponds in Mecca, CA one day after stocking *G. affinis*.

understanding of the ecological implications of introducing a biological control agent. Cultural control methods, such as source reduction, are generally used in conjunction with biological controls and mosquitocides in habitats that support extensive stands of vegetation and are continuously flooded. For intermittently flooded habitats such as rice fields, comparatively deep irrigation canals provide a refuge for mosquitofish when rice fields are drained and a supplemental source of fish to stocking when the rice fields are flooded. The efficacy of mosquitofish as mosquito control agents declines in intermittently flooded rice fields (Lacey and Lacey 1990), particularly in nursery rice fields (Prasad et al. 1993). Whereas mosquitofish can provide effective and persistent suppression of a variety of mosquito species (Swanson et al. 1996), *G. affinis* and *G. holbrooki* have not been not effective mosquito control agents in all habitats to which they have been introduced (Rupp 1996, Gratz et al. 1996). Mosquitofish work well controlling mosquitoes in drainage ditches, fish ponds, and other small water bodies (Mesich 1985, Scott 2006). Downs (1991) concluded that mosquitofish effectively control mosquitoes in a variety of natural and man-made (e.g., dairy, industrial, sewage, ornamental) ponds, ditches, and drains including underground and storm drains, permanent and seasonally flooded wetlands, water troughs, and non-chlorinated swimming pools.

Operational Considerations: Biotic Factors Affecting Efficacy

Mosquitofish are a particularly effective mosquito control agent in man-made habitats that either lack or contain sparse vegetation but can be subjected to high mortality from piscivores and cannibalism in open water. Piscivorous birds such as ardeiids (herons and egrets: Britton and Moser 1982) can cause significant mortality of mosquitofish populations in shallow habitats (< 20 cm depth), especially after stocking (Walton and Mulla 1989; Fig. 3). Herons and egrets preferred female mosquitofish to male *G. affinis* and shifted the sex ratio in some natural populations towards males (Britton and Moser 1982). Diving ducks such as mergansers can cause > 50% mortality in mosquitofish populations overwintering in open ponds (Schon 1997). Piscivorous fish [e.g., gar (*Lepisosteus* spp.), bass (*Micropterus* spp.), green sunfish *Lepomis cyanellus* R.] also can be detrimental to mosquitofish populations in sparsely vegetated habitats (Hunt 1953; Blaustein 1989b, 1992). Davey and Meisch (1977) suggested that mosquitofish and green sunfish can be stocked concurrently in rice fields because the green sunfish offers initial mosquito control that is better than *Gambusia* but mosquitofish reproduce rapidly in habitats where the sunfish die out. The compatibility of the 2 fish species in comparatively permanent habitats is probably much lower than in rice fields because large-sized piscivorous green sunfish are present and consume *Gambusia*. Negative interactions between *L. cyanellus* and *G. affinis* also were evident in some rice fields; however, the negative interaction between the 2 species was not always unidirectional (Blaustein 1992). Predaceous insects [e.g., notonectids (*Notonecta* spp.), water bugs (*Belostoma* spp. and *Lethocerus* spp.), large dytiscid beetles (*Cybister* spp. and *Dytiscus* spp.)] can also exert strong predation pressure on fish populations in some habitats (Wilson 1958, Coykendall et al. 1991, personal observation).

Provided that mosquitofish are not subjected to high levels of predation from piscivores and to extreme environmental conditions, the effectiveness of *Gambusia* spp. against mosquitoes in sparsely vegetated habitats is greater than in habitats containing thick vegetation (Collins et al. 1983, Walton and Mulla 1989, Orr and Resh 1991, Malhotra and Prakash 1992). Malhotra and Prakash (1992) found that adding *G. affinis* and vegetation management (removing vegetation from the periphery of small ponds and cutting the margins vertically followed by mud-plastering to limit regrowth of vegetation) markedly reduced the presence of *Anopheles* spp. and *Cx. quinquefasciatus*. Survival of mosquitofish fry is enhanced by shelter provided by vegetation; however, efficacy of mosquitofish as a biological control agent usually declines in vegetated habitats such as wetlands where the physical structure provided by vegetation decreases detection of immature mosquitoes by fish (Coykendall 1980, Schaefer and Miura 1985, Orr and Resh 1987, Berkelhamer and Bradley 1989). Thick vegetation also may limit access of fish and create conditions (e.g., low dissolved oxygen concentration) that inhibit fish movement into the center of vegetated zones.

Moreover, the types and density of vegetation influence the efficacy of mosquitofish as mosquito control agents. Submerged vegetation is thought to provide favorable habitat for mosquitofish population growth whereas floating and

surface vegetation reduce the efficacy of mosquitofish as a biological control agent for mosquitoes (Swanson et al. 1996). Decaying lodged emergent vegetation interfered with fish predation and enhanced larval mosquito survival, immature development and emergence success (Berkelhamer and Bradley 1989). Mosquitofish catches in submerged plants [*Ranunculus aquatilis* (Thuill.) DC] were higher than in the interior of stands of some emergent macrophytes (*Typha* spp.) in a riverine wetland (Keiper et al. 2003). Submerged vegetation with finely dissected leaves that contacts the water surface can provide shelter for mosquito larvae and significantly reduce predation by mosquitofish (Curtin et al. 1984, Orr and Resh 1987). Meisch (1985) summarized the findings of important studies such as Hildebrand (1919), Valle (1928) and Krumholz (1948) that discussed the vegetation-related factors affecting the efficacy of *Gambusia* for controlling vectors of malaria. In contrast to the negative effects of the physical structure of vegetation on larvivorous fish predation on mosquitoes, some studies (Angerilli 1980, Linden and Cech 1990) suggested that the dark background provided by vegetation enhanced the visibility of mosquito larvae to predacious fish.

Mosquitofish, as well as nearly any planktivorous fish species (Menon and Rajagopalan 1978, Wu et al. 1987), will reduce mosquito densities in vegetation-free habitats such as water storage reservoirs and cisterns (Sharma and Rajagopalan 1986); but starvation of fish is likely in smaller container habitats such as troughs. Larvivorous fish are not suitable for the control of mosquitoes that utilize small water containers, tires, etc. (Hurst 2004). Because fish can impart taste and oil into water and feces are visible in water, larvivorous fish are less preferred biological control agents in potable water supplies than are other less conspicuous biological agents such as predacious copepods (Fletcher et al. 1992, Lardeux 1992, Lardeux et al. 2002; B. H. Kay, Queensland Medical Institute, personal communication).

The success of biological control programs against mosquitoes using any predatory organism will depend on the functional and numerical responses of the predator population. The functional response of mosquitofish measures per capita prey consumption as a function of prey density and is influenced by mosquitofish size, prey size, prey density, the densities of alternative prey, the relative preferences of the predator for different prey, and other factors. The numerical response of the predator is the ability of the predator to convert prey into more predators. Following stocking, the larvivorous fish population has to grow and maintain sufficient numbers to exert significant mortality against the target pest. An ideal biological control agent should be stenophagous and be able to track closely changes in the pest species abundance. For biological control agents such as larvivorous fish that cannot migrate overland between aquatic habitats, larvivorous fish should prefer to prey on the target pest species yet be capable of maintaining itself on alternative prey if the target species populations are low seasonally.

Advantageous characteristics of mosquitofish for biological control include habitat preferences that overlap with pest species, broad environmental tolerances, feeding behavior, high reproductive rate, bearing live young, and amenability to culture and stocking operations (Swanson et al. 1996). Mosquitofish prefer warm, shallow, tranquil environments with emergent and/or submerged vegetation; immature mosquitoes are found in greatest abundance in these types of habitats when fish are not present. Small body size facilitates mosquitofish movement into many vegetated habitats that are preferred by the immature stages of many mosquitoes (Armstrong 1977). The strongly upturned orientation of the mouth facilitates feeding at the water surface where mosquito larvae are often found. Mosquitofish are omnivores with catholic diets (Hess and Tazwell 1942, Harrington and Harrington 1961, Washino and Hokama 1967, Bence 1985), but concentrate feeding on animal prey including terrestrial prey caught in the surface film (Farley 1980, Crivelli and Boy 1987), surface-dwelling insects (e.g., Collembola: Miura et al. 1979) and often on mosquitoes (Fleming and Schooley 1984, Linden 1986, Morton et al. 1988, Linden and Cech 1990). Even though the average clutch size and lifetime fecundity of a live-bearing fish such as the mosquitofish (Stearns 1983a, 1983b; Reznick and Miles 1989) is lower than that for egg-laying species (Cech and Linden 1987), mosquitofish are better suited as a biological control agent for mosquitoes than are most egg-laying fishes because *Gambusia* spp. mature quickly and females are capable of producing multiple broods during the period when mosquitoes are present. The ability to produce live young and not to require nesting enhances the numerical response of stocked populations and facilitates their use in eutrophic and hypereutrophic environments where hypoxia limits nesting activities and survival of eggs of other potential larvivorous fishes (Walton et al. 1996, 1997). Lastly, seasonal reproduction and population cycles of mosquitofish often overlap with those of mosquitoes in permanent and semi-permanent habitats (Swanson et al. 1996).

Meisch (1985) made the following 4 generalizations about the effectiveness of *Gambusia* against mosquitoes: (1) mosquitofish are generally more effective against permanent water mosquitoes than against floodwater mosquitoes. Because mosquitofish population size needs to

increase above that for standard practical stocking rates to exert significant levels of mortality on mosquito populations inhabiting many habitats, *Gambusia* populations are rarely large enough to control the synchronous and massive emergence of floodwater mosquitoes. (2) Mosquitofish appear to control culicines better than anophelines. (3) Mosquitofish efficacy is enhanced in bodies of water with little or no vegetation. (4) Juvenile mosquitofish are seldom as effective larval mosquito predators as are mature mosquitofish.

The orientation of anopheline larvae parallel to the water surface as well as the spatial distribution of anopheline larvae in mats of algae or within the meniscus surrounding emergent vegetation (Hildebrand 1919, Hess and Hall 1943) decreases the likelihood of detection by mosquitofish. Because of orientation at the water surface (e.g., larvae hang downward at an acute angle from the water surface) and diving behavior, *Culex* larvae have a greater probability of being detected by mosquitofish than do anopheline larvae. Several authors noted that anopheline larval numbers were reduced appreciably after *G. affinis* developed search images for anopheline larvae (Hess and Tarzwell 1942, Blaustein and Karban 1985). Hadjinicolaou and Betzios (1973) suggested that the introduction of *G. affinis* was effective against *Anopheles* mosquitoes that utilize permanent breeding places in Greece. Comparisons of the larval and adult densities before and after the introduction of mosquitofish revealed 1–2 orders of magnitude decline in adult *An. sacharovi* Favre abundance in collections, elimination of *An. sacharovi* larvae from developmental sites containing larvivores and a decline in malaria transmission, even in the absence of larviciding and any antimalaria measures. Mahmoud (1985) concluded that *G. holbrooki* was not an efficient control measure against *An. arabiensis* (Patton) during the peak season of malaria transmission in central Sudan because fluctuations in water flow in the irrigation canals were detrimental to mosquitofish, water control structures were often impediments to fish movement among canals, and alternative developmental sites for the mosquito were found outside the canal system during the rainy season.

Juvenile mosquitofish are seldom as effective larval mosquito predators as are mature mosquitofish. Not only do juvenile mosquitofish eat fewer mosquitoes per unit time than do adult fish, the diet breadth of juveniles is narrower than the adults (Farley 1980, Wurtsbaugh et al. 1980, Mansfield and McArdle 1998). Crivelli and Boy (1987) found that the diets of males and immature fish overlapped considerably (61.9–95.6%) and differed from the diets of mature females, especially during the breeding season. Because of limitations imposed by mouth size, juvenile mosquitofish usually do not prey on late stage larvae and pupae (Wurtsbaugh et al. 1980, Homski et al. 1994). Stocking juvenile fish is not as effective as stocking reproductive females because juvenile fish are less hardy than are adults and mature female mosquitofish can produce offspring sooner after stocking than would be possible by juveniles that require a period (~ 35–42 days: Krumholz 1948, Hubbs 1996) of maturation. However, Bence (1988) suggested that juvenile mosquitofish account for a large proportion of the mosquito mortality.

Other biological factors that affect the degree of mosquito control provided by *Gambusia* spp. include population size and structure, distribution in the habitat, availability of alternative prey and mosquito utilization of the source habitat (Swanson et al. 1996). The size of individuals, proportion of adult fish in the population, and sex ratio of the population will influence the feeding rate and predation pressure impinging on particular prey species. The combination of developmental changes in foraging ability (i.e., prey size and feeding rate increase during mosquitofish development) and the increasing number of fish primarily explain the onset of mosquito control observed after stocking. Female *Gambusia* have feeding rates higher than males (Bouallam et al. 1997, Cabral et al. 1998). Following stocking under favorable conditions for population growth, 2–6 wk are usually required for mosquitofish populations to reach a size effective for exerting mosquito control (Swanson et al. 1996). Dispersal of mosquitofish from habitats targeted for mosquito abatement may reduce fish population density and can lead to resurgence of mosquito production. Migration is usually greatest at 1–2 months after the onset of reproduction and newly mature females migrate in the largest numbers (Farley and Younce 1979, Coykendall 1980, Robbins et al. 1987). Factors such as water source, habitat age, water permanence, density of vegetation and other mosquito predators (Orr and Resh 1991, Walton et al. 1991, Beehler and Mulla 1993, Ritchie and Laidlaw-Bell 1994, Angelon and Petranka 2002) influence the attractiveness of habitats to gravid mosquitoes and survival of immature mosquitoes. Not surprisingly, the density of fish required to suppress mosquito populations varies directly with the propensity to produce mosquitoes among habitats (Swanson et al. 1996).

Operational Considerations: Abiotic Factors Affecting Efficacy

Mosquitofish are comparatively hardier than are other fish species that have been considered for biological control of mosquitoes (Bay 1985, Gerberich and Laird 1985); nevertheless, environmental conditions can affect the success of *Gambusia* for mosquito control. Physicochemical

Table 2. Tolerance of *Gambusia* spp. and suggested values for selected water quality parameters in mosquitofish culture (from Swanson et al 1996).

Water quality factor	Tolerated[a]	Suggested	Optimal[b]
Temperature[c]			
(°C)	0.5–42	10–35	25–30
(°F)	33–108	50–95	77–86
Dissolved oxygen[d] (mg/L)			
with access to surface	0.2–>35	3–12	5–12
with no access to surface	1.3–>35		
pH	4.65–>10.2	7.0–9.0	7.5–8.2
Ammonia (mg/L NH_3-N)	0–1.0[e]	0–0.1	as low as possible
Nitrite (mg/L NO_2-N)	unknown	0–0.5	as low as possible
Salinity (g/L or ‰)	0–58	0–25	0–12

[a] LC_{50} or lowest/highest level in which survival reported.
[b] for maximal survival, growth and reproduction
[c] at approximately 100% saturation of oxygen
[d] at 25°C
[e] Multiply by 1.25 to calculate the ammonia concentration [NH_3].

factors such as water temperature, pH, dissolved oxygen and the presence and concentration of chemical pollutants are particularly important determinants of the success of biological control programs using mosquitofish (Swanson et al. 1996; Table 2). Mosquitofish can tolerate a wider range of water temperature than do some alternative fish species for mosquito biocontrol (Otto 1973, Al-Habbib and Yacoob 1993, Offill and Walton 1999) but growth is optimal in a comparatively narrow range of temperature (Wurtsbaugh and Cech 1983, Downs and Beesley 1983; Table 2). Mosquitofish can tolerate low dissolved oxygen concentrations by residing at the water surface (Odum and Caldwell 1955, Lewis 1970, Cech et al. 1985, Hubbs 2000) but under such conditions susceptibility of fish to avian piscivores must increase appreciably. Like temperature tolerance, the ranges of pH and salinity tolerated by *Gambusia* are considerably wider than should be found in habitats for successful biological control programs and culture systems (Table 2). The metabolic capacity of adult female *G. affinis* varied little in 0, 10 and 20‰ salinity (Akin and Neill 2003). Even though *G. affinis* can withstand brief periods of transfer from freshwater to 50% sea water, mortality increased abruptly and significantly when fish were transferred from freshwater to full strength sea water for > 24 h (Salibian 1977, Al-Daham and Bhatti 1977).

Mosquitofish are very sensitive to nitrogenous compounds such as unionized ammonia and nitrite (Table 2) that are present in most wastewaters and can occur at toxic or stressful levels when mosquitofish densities are high, especially during transport and aquaculture. The susceptibility of *Gambusia* to stresses of ammonia toxicity is however not significantly greater than for other fishes considered for biological control of mosquitoes. Beesley et al. (1985) found that mosquitofish survival in 21-day rearing studies was higher than for other fish species (compared with survival of salmonids and ictalurids cited in publications) at similar levels of ammonia (range across 2 stocking rates: 0.010–2.81 mg/liter) and nitrite (range across 2 stocking rates: 0.030–0.990 mg/liter) and speculated that growth rate would be zero at 0.7–0.8 mg NH_3 per liter and 0.15 mg NO_2 per liter. The LC_{50} values for *G. affinis* exposed to unionized ammonia for 24–96 h ranged from 0.72–0.67 mg/liter (range of [NH_3-N] = 60–51 mg/liter; Sangli and Kanabur 2001). Chronic effects of ammonia toxicity are probably evident at ammonium nitrogen concentrations as low as 0.1 mg/liter; Swanson et al. (1996) recommended that NH_4–N concentrations in culture systems do not exceed this value. Microbial metabolism associated with high loading rates of ammoniacal and organic nitrogen will decrease dissolved oxygen concentration in water; potentially toxic levels of unionized ammonia for *Gambusia* can be attained rather quickly during diel changes in pH at temperatures commonly observed in nature during the reproductive period. Ammonia tolerance differs seasonally as a function of temperature acclimation and other factors. Warm-water aquatic communities were 1.5–6 times more sensitive to the toxic effects of ammonia at cold versus warm temperatures (Nimmo et al. 1989).

In addition to potential ammonia toxicity, endocrine disruptors and halogenated compounds in wastewater can pose significant detriments to larvivorous fish populations. Hermaphroditism has been recorded in mosquitofish populations subjected to masculinization factors in pulp-mill effluents (Howell et all. 1980, Cody and Bortone 1997) and from unknown causes under laboratory colony rearing (Teh et al. 2000).

Table 3. Mosquitofish stocking rates and dates in selected mosquito habitats (modified from Swanson et al 1996).

Habitat	Stocking rate[a] kg/ha (lb/acre)	Stocking date[b]	
Ponds	0.56–1.12 (0.5–1.0)	April–Sept.	
Marshes Sloughs Pastures	0.56–1.12 (0.5–1.0)	April–Sept.	
White rice fields	0.22–0.67 (0.2–0.6)	April–Sept.	Hoy and Reed 1970 Hoy et al. 1971, 1972
Wild rice fields	1.1–3.4 (1.0–3.0)	June–Sept.	Kramer et al. 1988
Rice fields: intermittent flooding (AR)	2.3–5.7 (2.0–5.0)		Meisch and Coombes 1974
Polluted/stagnant waters	0.56–1.68 (0.5–1.5)	April–Sept.	
Duck club ponds	1.4–4.0 (1.2–3.6)	Aug.–Nov.	Walton and Mulla 1989
Shaded cool waters	0.56 (0.5)	March–June	
Sunlit cool waters	0.56 (0.5)	Nov.–April	
Underground drains	2.82 (10)[c]	June	Mulligan et al. 1983

[a] Stocking rate is a function of date and mosquito density.
[b] The range of stocking dates reflects differences in location and target mosquito species.
[c] kg/km (kg/mile)

Feminization has been recorded in *Gambusia* populations residing in sewage-contaminated water (Batty and Lim 1999). Male reproductive capacity was impaired in a Florida lake contaminated with antiandrogenic compounds (Toft et al. 2003).

Residual chlorine in water and the interaction of disinfection byproducts with ammoniacal nitrogen and wetland-derived organic matter can create toxic conditions for fish and other aquatic organisms in constructed treatment wetlands where NH_4–N levels are elevated. If dechlorination of the influent water to a treatment wetland is not fully effective or the influent water supply is contaminated by chlorinated water, then chlorine and halogenated compounds (e.g., chloramines, trihalomethane or other disinfection byproducts; Nimmo et al. 1989, Rostad et al. 2000) can enter a constructed treatment wetland. Even though the concentrations of potentially toxic disinfection byproducts released from water treatment plants decline as water moves through constructed treatment wetlands, enhanced concentrations of wetland-derived organic carbon (Barber et al. 2001) are thought to increase dramatically the potential for toxic effects ($\sim$ 30-fold) from disinfection byproducts (Rostad et al. 2000).

Seasonal changes in mosquitofish reproduction are influenced by photoperiod (Sawara 1974, Reznick and Braum 1987) and reproduction may be impeded in dark habitats such as underground storm drains. Mulligan et al. (1983) found that *G. affinis* provided reductions of 75–94% of *Cx. quinquefasciatus* relative to drains without mosquitofish during a 14-wk period in Fresno, CA. Female mosquitofish that were gravid at stocking produced young, but no mating of fish was observed in the drains. In addition to a failure to mate, the relative condition of the fish population declined as food levels in the drains declined during the summer (Mulligan et al. 1983).

Stocking Larvivorous Fish

The stocking rate of mosquitofish depends on the type of mosquito developmental site, time of year, and the condition and composition of the fish being stocked. Stocking rates are comparatively high under the following conditions: (1) sites where immediate control is required such as sites supporting floodwater mosquitoes (Todd and Giglioli 1983, Meisch 1985), (2) habitats such as seasonally flooded duck hunting club wetlands (Walton and Mulla 1989, 1991) flooded late in the season when mosquitofish reproduction declines naturally, (3) habitats that are poor quality for fish such as municipal or agricultural wastewaters containing high levels of ammonium nitrogen or biochemical oxygen demand, and (4) habitats that support high immature mosquito abundance (Table 3). The objective of stocking programs is to release the minimum number of fish at the time when conditions in the habitat promote rapid predator population growth and at locations facilitating dispersal within the water body of concern (Swanson et al. 1996). Whereas mosquitofish tend to move downstream from release sites (Green and Imber 1977, Reed and Bryant 1974, Farley and Younce 1978, Coykendall 1980), multiple release sites usually result in a mosquitofish distribution that is less aggregated than for single release points in large water bodies (among paddies in 12–38 ha rice fields: Farley

Table 4. Conversions for wet weight into number of mosquitofish.

Population composition	Conversion rate		References
	number/kg	number/lb	
Mixed population (juveniles and adults)	1,300–2,900	600–1,300	Hoy et al. 1972 Davey et al. 1976 Meisch 1985
Mature females	1,100	500	Downs 1991
Mean	2,200	1,000	Swanson et al. 1996

and Younce). Swanson et al. (1996) recommend a minimum of 3 releases at several locations in large, geographically complex habitats. Geographic barriers to movement such as dikes and large areas of open water will slow the movement of mosquitofish between habitats. Stocking rates in floodwater habitats are 4–10 times greater than for ponds (Table 3).

Stocking rates are often defined as wet weight of mosquitofish per unit area or the number of fish per unit area. The number of mosquitofish per unit weight depends on a variety of factors such as the relative abundance of juveniles versus adult fish in the sample, the time of year, and the source of the fish (i.e., wild-caught vs. cultured). There are between 1,300 and 2,900 individuals per kg (Table 4). Provided that immediate mosquito control by *Gambusia* is not needed, mosquitofish should be released 1–3 wk after flooding (Swanson et al. 1996) because the availability of food and shelter for mosquitofish is typically low in newly inundated habitats. Supplemental control measures such as bacterial larvicides are often needed in habitats flooded late in the year when mosquitofish reproduction declines naturally (Walton and Mulla 1989) and may be used early in the season if mosquito production is high enough to warrant control before mosquitofish populations are large enough to exert control (Walton and Mulla 1991). Mosquitofish density in California rice fields was estimated to be 11 fish per m^2 at season's end (Stewart and Miura 1985) – similar to mosquitofish densities of 16 fish per m^2 in experimental rice paddies where significant levels of mosquito control were observed in 1 of 2 studies (Cech and Linden 1987). Much lower densities (0.5–5 *Gambusia* per m^2) were required for control of mosquitoes in Georgian reservoirs; fish density depended on the extent of vegetation growth and the periodic cleaning during the season (Maruashvili and Sichinava 1985).

To fulfill the criteria for safe and effective use of mosquitofish for biological control programs against mosquitoes outlined by Swanson et al. (1996), a mosquitofish stocking guide could be used to assist decision- making by field staff and to supplement training in operational procedures that facilitate compliance with state and regional regulations for stocking fish. Legner and Sjogren (1984) suggested that a comprehensive view of biological control should differentiate geographic areas, habitat characteristics and levels of human tolerance, especially as these factors relate to the severity of public health concerns. Table 5 illustrates a mosquitofish stocking guide used by the Contra Costa VCD (Concord, CA). The conditions in habitats commonly producing mosquitoes are used to define a matrix of stocking decisions. A similar guide could be developed for mosquito developmental sites and larvivorous fish stocking rates in other regions.

All conditions in a habitat must be favorable for fish stocking to proceed; however, 2 decision dichotomies (Table 5) require clarification: the presence of mosquito predators and the water velocity that distinguishes low flow from high flow. All aquatic habitats are likely to support insect predators of mosquitoes. If the stocking decision were based on the presence of any potential mosquito predator then larvivorous fish would never be stocked; this decision dichotomy should be based on the presence-absence of either native vertebrate larvivores or aquatic insects such as *Notonecta* spp. that have demonstrable effects on immature mosquito abundance.

The larvae of most mosquito species associated with lotic environments are found in pockets of still water along the periphery of streams where conditions should be favorable for mosquitofish and most larvivorous fishes. Larvae of the few culicid species found in running waters inhabit the boundary layer adjacent to objects penetrating the water surface or use modified setae to cling to surfaces (Clements 1999). Clements' (1999) summary of the scientific literature addressing the responses of immature mosquitoes to water movement indicates flows $>$ 7.6 cm/sec flushed larvae anchored to objects (threshold flow for flushing of larvae: *An. minimus* Theobald: 7.6 cm/sec; *An. aconitus* Doenitz $>$ 6.7 cm/sec; *An. hyrcanus* Pall. $>$ 5.5 cm/sec). Such flow rates are well within the swimming abilities of mosquitofish as indicated in Ward et al. (2003). Predatory efficiency of *G. affinis* in a mud-pot decreased in running water as both the distribution of mosquito larvae (*An. stephensi* Liston or *Cx. fatigans* Wied.) and the behavior of the fish changed in flowing water; predation rate of female mosquitofish in water flowing at 0.8 ml/sec did not differ appreciably from that in

Table 5. Mosquitofish stocking guide used by the Contra Costa Vector Control District (Concord, CA). DS = do not stock mosquitofish; S = stock mosquitofish; NA = not applicable

	Source[b]									
Conditions[a]	Lake or pond	Creek	Horse trough or wine barrel	Swimming pool or ornamental pond	Irrigation ditch	Marsh	Catchment basin	Flood control canal	Duck club pond	Rice field
Threatened or endangered species present (aquatic)[b]	**DS**†	**DS**	**NA**	**NA**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**
Threatened or endangered species absent (aquatic)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Immature mosquitoes present (or historically present)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Immature mosquitoes absent	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**
Mosquito predators present	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**
Mosquito predators absent	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Water temperature below 55°F (13°C)	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**
Water temperature above 55°F (13°C)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Salinity below 15‰	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Salinity above 15‰	**NA**	**NA**	**NA**	**NA**	**NA**	**DS**	**NA**	**NA**	**DS**	**DS**
Distance to populated area less than 10 mi (16 km)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Distance to populated area greater than 10 mi (16 km)	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**	**DS**
Water velocity high	**NA**	**DS**	**NA**	**NA**	**DS**	**NA**	**DS**	**DS**	**NA**	**NA**
Water velocity low	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Recommended number of mosquitofish (lbs./acre)+	**0.5–1.0**	**0.5–1.0**	**3–20 fish**	**25–100 fish**	**0.5–1.0**	**0.5–1.0**	**25–100 fish**	**0.5–1.0**	**1.2–3.6**	**0.2–3.0**

[a] All conditions must be favorable for fish stocking to proceed.
[b] Consult appropriate staff if the status or endangered species is unknown or if there is a potential for fish to migrate from the stocking site.
† Stocking can be conducted if it is determined that the threatened or endangered species will not be affected.
+ Stocking rates adapted from Swanson et al. (1996).

standing water whereas predation rates when water was flowing at 2.4 and 10.2 ml/sec were 60% and 30%, respectively, that in standing water (Reddy and Pandian 1974). Without knowing the volume of water in the mud pot and relative volume exchanged under the aforementioned flow rates, it is difficult to make a recommendation for flow rate of high vs. low flow. Based on Congdon's (1994) study, flows $\leq$ 1 cm/sec would be considered low.

Stocking both mosquitofish and another fish species has been suggested to augment predation on immature mosquitoes by either enhancing fish numbers early after stocking before mosquitofish populations can increase to effective levels or focusing predation elsewhere in the water column besides the water surface (Schooley and Page 1984). The threespine stickleback (*Gasterosteus aculeatus* L.) was inferior to *G. affinis* for controlling mosquitoes in earthen ponds and combining the 2 species did not enhance mosquito control relative to mosquitofish alone (Offill and Walton 1999). Homski et al. (1994) recommended repeated applications of 2 fish species (*Aphanius dispar* Rüppell (Cyprinodontidae) and *G. affinis*) for biocontrol of mosquito larvae in sewage-contaminated and other low quality waters in Israel but it is hard to see that such an approach would be cost-effective. Cech and Linden (1987) found that combining highly fecund, early reproducing Sacramento blackfish (Cyprinidae: *Orthodon microlepidotus* Ayres) with mosquitofish (*G. affinis*) in small rice paddies did not significantly enhance mosquito control compared to mosquitofish alone. Moreover, larval mosquito abundance after 12 wk in paddies stocked with only blackfish was higher than in paddies without fish; larval mosquito numbers in the high stocking density (30 fish per 0.01 ha) mosquitofish alone treatment were lower than in the controls but did not differ significantly. Mosquitofish outperformed blackfish and hitch (*Lavinia exilicauda* Baird and Girard) in other experiments in rice paddies (Cech and Linden 1986). Bellini et al. (1994) found that *Carassius auratus* (L.), *Cyprinus carpio* (L.) and *G. affinis* did not have a significant impact on mosquito abundance in rice fields of northern Italy. In saltmarshes in eastern Australia, *G. affinis* co-occurred with native fishes (*Pseudomugil signifier* Kner and gobiids) and all fish species consumed mosquito larvae in large numbers when mosquitoes were dominant but were unable to provide significant control of *Aedes vigilax* (Skuse) in the highly variable saltmarsh environment (Morton et al. 1988).

Monitoring Stocked Populations

Regular monitoring of mosquitofish populations should include assessments of fish density, population growth and biocontrol efficacy (Swanson et al. 1996). Aerial samplers are ineffective for sampling *Gambusia* (Takahashi et al. 1982). Mosquitofish populations are frequently monitored using minnow traps. There are several designs for minnow traps including flexible mesh, bottles, and hardware cloth cages (Fig. 4). All designs incorporate funnel-shaped features that concentrate fish inside the trap and inhibit escape. Blaustein (1989a) noted that unmodified wire minnow traps trapped large-sized mosquitofish ($\geq$35 mm total length) with greater efficiency than for small-sized fish. The catch of small-sized fish by minnow traps constructed of hardware cloth (Gee's Improved Wire Minnow Trap®, Cuba Specialty Mfg, Co., Fillmore, NY) is enhanced by lining traps with fiberglass window screen. Traps should be set so that a portion of the trap is above the water surface to intercept the surface-dwelling fish and to provide sufficient oxygen for the trapped fish. The latter consideration is particularly important in hyper-eutrophic environments where low dissolved oxygen concentration below the water surface would be lethal to the fish. When traps are deployed in water with a depth greater than the cross-sectional diameter of the minnow trap, floats can be inserted to maintain a portion of the trap above the water surface. For example, a 21.5 cm (8.5 in.) section of children's swimming pool float (FunNoodles®; Nomaco Inc., Zebulon, NC) is sufficient to support a wire minnow trap (Fig. 4).

Minnow trap catches provide an estimate of relative population size that is sufficient for monitoring stocked mosquitofish populations in most biological control programs. Minnow traps are a passive sampling device and trap catches rarely provide a meaningful measure of absolute fish population density; however, the number of fish captured in minnow traps has been positively correlated with the total number of fish in the environment (Reed and Bryant 1975, Norland and Bowman 1976, Miura et al. 1982, Stewart and Miura 1985). Measurement of absolute population density is difficult in most large mosquito developmental sites because mosquitofish are not distributed uniformly throughout sites, the fish often move around the habitat in a contagious dispersion pattern (e.g., schooling or shoaling), and trap efficiency is not constant as mosquitofish population size changes. Minnow trap catches are influenced by environmental factors, time of day, fish population structure, and trap location (Swanson et al. 1996); therefore, trapping locations and the period of trap deployment should be consistent on different dates when trap counts are to be compared across time. Traps should be placed in different habitats within large field sites such as field centers and perimeter zones of rice fields (Norland and Bowman 1976, Lacey and Lacey 1990). Minnow

Fig. 4. Minnow trap-style sampling devices for collecting mosquitofish. Gee's Improved Wire Minnow Trap® lined with fiberglass window screen (upper panel) and deployed (inset upper panel). Three alternative minnow trap designs (lower panel left to right: Camp-style plastic minnow trap, Camp® glass minnow trap, and the BioQuip® aquatic (#2821) trap).

traps are often deployed for 24 h. The presence of mosquitofish in a minnow trap (Norland and Bowman 1976) or vegetation density and type (Blaustein 1989a) did not influence trap capture efficiency; however, trap capture efficiency was influenced by water depth (Blaustein 1989a). Other collection methods include seines, cage traps and pop-up traps.

Biocontrol efficacy can be assessed using standard sampling procedures for mosquitoes. Service (1993) provides an extensive discussion of sampling methodologies and the measurement of mosquito populations.

NEGATIVE ASPECTS OF USE

Several risks and drawbacks associated with the use of *Gambusia* for mosquito control were mentioned by Rupp (1996) and Gratz et al. (1996) including (1) a lack of efficacy in some habitats, (2) mosquito control that is not significantly better than that provided by with native species, (3) negative impact on native fishes and biota, (4) reduction of non-target aquatic invertebrates that may be important natural enemies of mosquitoes and important components of integrated control strategies and (5) adverse food web effects such as algal blooms that occur after reduction of herbivorous plankton by mosquitofish (Hurlbert and Mulla 1981, Nagdali and Gupta 2002). Service argued that an effective control agent must reduce biting densities of the adult mosquitoes and/or reduce disease prevalence (Gratz et al. 1996). Reductions in densities of immature mosquitoes by larvivorous fish are presumably offset by compensatory beneficial effects to the mosquitoes of development at reduced density. Despite being introduced in about 60 countries and claims that malaria transmission was reduced in parts of Afghanistan, China, Ethiopia, Iran, Korea, Somalia, Turkey, and the Ukraine, successful control programs using mosquitofish have been limited to

> "discrete more or less permanent larval habitats such as water reservoirs in dry area, control has been very local, and there is usually no evidence of sustained disease control."

However, Swanson and Cech argued that many of the

> "examples of biocontrol failure can be attributed to ignorance of mosquitofish biology and of the ecology of the stocked aquatic system, and resultant stocking of mosquitofish into inappropriate habitats and/or use of inadequate or incorrect operational methods" (Gratz et al. 1996).

They suggested that

> "the objective of mosquito control using mosquitofish should be to select and apply the fish in those specific circumstances where the possibility of control is greatest and adverse impacts on the aquatic system are minimized."

Mosquitofish have not controlled mosquitoes in all habitats to which they have been introduced and failures to plan properly for introduction and use have created problems (Meisch 1985). Omnivorous behavior and broad diet preferences contribute to the mosquitofish's success for providing long-term mosquito control in habitats where mosquito larvae may be present only periodically. A dependence on *Gambusia* as the sole mosquito control agent in large water bodies (e.g., rice fields, marshes, irrigated pastures, constructed treatment wetlands), especially those containing thick vegetation cover and abundant food, is inadvisable.

The same qualities that make mosquitofish well-suited for biological control of mosquitoes in a variety of habitats also make *Gambusia* spp. ideal invasive species. After introduction to habitats outside the native geographic range, *Gambusia* spp. are purported to have harmful effects on ecosystems and native fishes (WHO 1982, Lloyd et al. 1986, Arthington and Lloyd 1989, Courtenay and Meffe 1989, Lacey and Lacey 1990, Rupp 1996, Howe et al. 1997, Warburton and Madden 2003). More than 30 species of native fish have been adversely affected by the introduction of *Gambusia* through predation or their indirect impact through competition (Legner 1995). Seasonal differences in reproduction, an inability of the exotic species to aestivate and different diel activity patterns facilitated sympatry between the native black mudfish (*Neochanna diversus* Stokell; Galaxiidae) and the introduced mosquitofish (*G. affinis*) in wetlands in the Waikato region of New Zealand. The factors permitting persistence of *Poeciliopsis* with *Gambusia* differed in spring pools and in flowing water in Rio Yaqui drainage (Galat and Robertson 1992). In spring pools, *Poeciliopsis* exhibited a compensatory increase in reproductive output when it co-occurred with mosquitofish. In streams, coexistence of the Yaqui topminnow with mosquitofish was facilitated by recurrent flash flooding and a uniform temperature springhead that provided refuge for *Poeciliopsis* (Galat and Robertson 1992). Behavioral responses to high flows and not differences in physiological swimming ability presumably account for differential displacement of *G. affinis* over *Poeciliopsis occidentalis* in canyon-bound streams of the southwestern United States (Meffe 1984, Minckley and Meffe 1987, Ward et al. 2003). Human-caused changes in environments such as changes in water quality and hydrology that may favor mosquitofish over native species cannot be definitively ruled out as significant factors in some instances where mosquitofish are thought to have replaced native species. For

example, in water bodies throughout Syria *G. holbrooki* is better adapted to high salinity and polluted water whereas the indigenous cyprinodont fish *Aphanius mento* (Heckel) tolerates stronger currents (Krupp 1992). Bay (1973) and Gerberich and Laird (1985) argued that the negative effects of *Gambusia* on the habitat of indigenous small fishes and food fishes are overrated.

Results of mostly laboratory-based studies also suggest mosquitofish reduce populations of amphibians (Grubb 1972, Gamradt and Kats 1996, Morgan and Buttemer 1996, Webb and Joss 1997, Goodell and Kats 1999, Baber and Babbitt 2004). Laboratory experiments indicate that the susceptibility to mosquitofish differs among anuran species (Komak and Crossland 2000) and that native fishes also consume susceptible amphibian species (Pyke and White 2000). Australian fishes [*Hypseleotris compressa* (Krefft), *H. galii* (Ogilby), *P. signifier* and *Perca fluviatilis* L.] preyed on susceptible amphibian species (e.g., the green and golden bell frog, *Litoria aurea* Lesson) albeit to a lesser extent than did *G. holbrooki* (Pyke and White 2000).

Results from well-designed field experiments do not always agree with the results from laboratory studies in aquaria, that showed the extirpation of some non-target species by *Gambusia* (Lawler et al. 1999). Some literature often cited to support the negative effects of *Gambusia* (Myers 1965) contains no original data, provides no quantitative evaluations to support claims of a lack of biocontrol efficacy (Downs, personal communication) or utilizes highly artificial experimental environments that are structurally simple or offer the fish limited prey selection. These same criticisms apply to some studies purporting to demonstrate the efficacy of mosquitofish as mosquito control agents. In natural populations, predation on mosquitofish, environmental complexity and environmental factors may ameliorate the strong effects observed in comparatively simple laboratory and mesocosm study systems (Haq et al. 1992, Krupp 1992, Belk and Lydeard 1994). Population censuses made at different times document that an introduction has occurred and that the faunal composition has changed between censuses but such correlational findings do not prove cause and effect and cannot identify the mechanisms causing the observed faunal changes (Courtenay and Meffe 1989). Nevertheless, it is ill advised to carry out introductions of non-native species in order to investigate the outcome of interactions with native species under natural conditions. Introduction of non-native fishes should be made with great caution and only after the undesirable effects of introduction are investigated and understood (Courtenay and Meffe 1989), regardless of the purpose (i.e., biological control, sport fishery, forage or food fishery) of the introduction.

ALTERNATIVES TO GAMBUSIA

A recent focus of many biological control programs has been to find an endemic larvivorous species as hardy, prolific and effective as *Gambusia* for mosquito control. This focus is hardly new as alternative fishes to the mosquitofish have been considered since the early 1900s (Bay 1973). Even if a species as hardy as *G. affinis* or *G. holbrooki* were found, it is highly unlikely the species would be considered for mosquito control outside its native geographic range. Bay (1985) stated that the characteristics of a fish that might replace the mosquitofish include (1) a general preference for mosquito larvae, (2) minnow size and (3) a rapid, high reproductive rate. In addition to these 3 criteria, a small-sized larvivore (adult size $<$ 6 cm TL) should exhibit a high degree of tolerance to salinity and pollution and should not be detrimental to non-target fauna in the aquatic ecosystem (WHO 1982). The socioeconomic characteristics of a locality merit consideration especially in places where pisciculture is an important community activity and incorporating fish species that are both effective larvivores and edible is necessary to ensure continued community participation in vector control programs (WHO 1982, Lacey and Lacey 1990).

Approximately 300 species of fish have been considered for mosquito control (Gerberich and Laird 1968, 1985; Ahmed et al. 1988; Legner 1995; Hurst 2004). Table 6 provides a simple rating system to help select fish species for mosquito control (Ahmed et al. 1988). Aplocheilids (formerly in the genus *Panchax*) in several Southeast Asian and African genera and the guppy, *P. reticulata*, were the foci of most published studies (Gerberich and Laird 1968). Bay (1985) suggested that species in the Cyprinidontidae are likely to be the most useful group of fishes for biological control. Representatives of the family occur in the temperate climates of every continent except Australia. The egg-laying tooth carps (Cyprinidontidae and Adrianichthidae) predominate in the Old World. The most promising genera include *Aphanius, Valencia* (Mediterranean region and western Asia); *Aplocheilus, Oryzias* (southern Asia); *Epiplatys, Aphyosemion, Roloffia* (West Africa); *Nothobranchius, Pachypanchax* (East Africa); *Rivulus, Fundulus, Cynolebias* and *Cyprinodon* (North and South America) (WHO 1982). Nelson and Keenan (1992) suggested the Plains killifish *Fundulus zebrinus* Jordan and Gilbert as an alternative to *Gambusia* spp. The inland silverside [*Menidia beryllina* (Cope): Atherinopsidae] did not survive well and did not consume mosquitoes

Table 6. Rating system for the selection of appropriate, indigenous fish species for mosquito control modified from the system developed by Ahmed et al. (1988). For each fish species under consideration, the relevant questions are answered and the total score is calculated. The fish with the highest score is the most likely candidate for the situation. E indicates eliminate the fish species from consideration.

1. Ability to withstand low dissolved oxygen (D.O.) levels	
a. Can live in water with D.O. approximately 0 mg/L	5
b. Can live in water with D.O. of 0 to 2 mg/L	3
c. Can live in water with D.O. of 3 to 5 mg/L	1
d. Can only live in water with D.O. > 5 mg/L	0
2. Ability to withstand high temperatures	
a. Can live at temperatures > 35°C	5
b. Can live at temperatures of 30 to 35°C	3
c. Can live at temperatures of 25 to 30°C	1
d. Can only live at temperatures < 25°C	0
3. Feeding habits (when direct larval predation is desired).	
a. Insectivorous, with strong preference for mosquito larvae	10
b. Insectivorous, midwater or surface feeder lacking strong preference for mosquito larvae	5
c. Insectivorous, mainly benthic feeder	3
d. Omnivorous	1
e. Herbivorous	0
4. Ability to penetrate vegetation	
a. Will penetrate thick stands of emergent/submergent vegetation	5
b. Will penetrate open stands of vegetation	3
c. Avoids vegetation	0
5. Reproductive rate in the field	
a. High, quickly produces large, self-sustaining population	10
b. Moderate	5
c. Low	3
d. Reproduces only in a subset of the habitats	1
e. Does not reproduce in mosquito habitats	0
6. Endemicity	
a. Native to area where control is desired and resides in the same watershed	10
b. Native to area where control is desired and reside in a different watershed	5
c. Native to zoograpahic region	3
d. Not native to area	0
7. Susceptibility to mass culture	
a. Easy to culture in large numbers	5
b. Culture possible, with some difficulty	3
c. Culture probably possible but difficult	1
d. Impossible to culture	0
8. Economics	
a. Cost low compared to other methods of control	5
b. Cost comparable to other methods of control	3
c. Cost high compared to other methods of control	0
9. Ease of transport	
a. Can survive extended periods of being crowded with minimal care	5
b. Moderately easy to transport	3
c. Difficult to transport far	0
10. Acceptibility for use in integrated control programs	
a. Highly acceptable, resistant to pesticides	5
b. Moderately acceptable	3
c. Difficult to use	0
11. Impact of the fish on native species	
a. Fish studied, no impact found	10
b. No information available	5
c. Suspected cases of extinction	1
d. Well-documented cases where the proposed agent caused extinction	E (0)

Table 6. Continued.

12. Impact of the fish on other mosquito larvae predators	
a. No effect on invertebrate predators	2
b. Minor impact on invertebrate predators	1
c. Fish reduces populations of invertebrate predators	0
13. Number (density of fish) needed for control	
a. Low numbers effect good control	5
b. Moderate numbers needed	3
c. Very high numbers of fish needed	0
14. Likelihood of human interference with the fish	
a. Fish unlikely to be harvested	5
b. Fish has high ornamental or food value	0
15. Potential for control during floods	
a. Fish will travel with flood waters, effective control	5
b. Fish resist moving with flood waters	0
16. Ability to withstand low temperatures	
a. Can overwinter at temperatures of 0 to 5°C	5
b. Can overwinter at temperatures of 5 to 10°C	4
c. Can overwinter at temperatures of 10 to 15°C	3
d. Can overwinter at temperatures of 15 to 20°C	1
e. Can overwinter at temperatures > 20°C	0
17. Tolerance to salinity and other minerals	
a. Can live in sea water (33‰)	5
b. Can live in strong brackish water (up to 20‰)	3
c. Can live in slightly brackish water (5 to 10‰)	1
d. Can only live in fresh water	0
18. Availability of the fish	
a. The fish can be collected in large numbers locally.	5
b. The fish can be collected in moderate numbers	3
c. The fish must be imported.	0
19. Ability to withstand physical disturbances in the environments	
a. Fish highly tolerant of disturbances	5
b. Fish moderately tolerant of disturbances	3
c. Fish intolerant of disturbances	0
20. Disease resistance	
a. Fish is highly resistant to disease in the area	5
b. Fish moderately resistant to local diseases	3
c. Fish is susceptible to local diseases	0
21. Government regulations	
a. There are no government regulations regarding use or importation of the fish	5
b. Following required permitting procedures will take some time and effort	3
c. Use of the fish is prohibited	E
22. Ability to survive dry spells	
a. Eggs are resistant to desiccation (annual fishes)	5
b. Species is not able to survive desiccation	0
23. Behavior of the fish in running water	
a. The fish aggregate in the shallows and backwaters.	5
b. The fish remain out in the current.	0
24. Ability to withstand pollution	
a. Highly resistant to polluted water	5
b. Can withstand moderately polluted water	3
c. Requires clean water	0
25. Tolerance to low pH	
a. Can live in very acidic water	5
b. Can tolerate moderately acidic water	3

Table 6. Continued.

c. Requires alkaline water	0
26. Larvae control by elimination of aquatic weeds	
a. The fish is known to consume the target weed species	10
b. The fish is known to consume other weed species	5
c. The fish is herbivorous, specifics unknown	3
d. The fish will not eat aquatic weeds	0

in wild rice fields in central California (Kramer et al. 1987). Potential biological control agents also are likely among the live-bearing groups including the Poeciliidae, Goodeidae, and Anablepidae (Anablepinae and Jenynsiinae).

Hurst (2004) summarized the results of laboratory feeding trails for 59 species of larvivorous fish and evaluated 7 fish species native to the Brisbane region of Queensland for mosquito biocontrol. The crimson-spotted rainbow fish [*Melanotaenia dublouyi* (Castelnau)] and the firetail gudgeon (*H. galii*) were identified as having the greatest potential as mosquito agents and the development of rearing facilities for the native fishes was encouraged (Hurst 2004). The use of alternative fish has often been restricted by inadequate supplies or the comparatively high cost of alternative species from commercial suppliers (Gratz et al. 1996).

Legner and Warkentin (1989) suggested that the desert pupfish, *Cyprinodon macularius* Baird and Girard, is an attractive substitute or supplement to *G. affinis* for mosquito control because it forages mostly in the benthos when mosquitoes are rare, forages effectively in vegetation, is effective at consuming mosquito larvae at comparatively lower densities than is *Gambusia*, and has broad salinity and temperature tolerances. Because the desert pupfish is protected by federal regulations it is doubtful that the fish would be introduced for mosquito control in habitats where source reduction is a component of an integrated pest management program within its native geographic region. Castleberry and Cech (1990) compared the efficacy of pupfish (*Cyprinodon nevadensis amargosae* Miller), mosquitofish (*G. affinis*) and guppies (*P. reticulata*) for mosquito control in sago pondweed marshes. All fish species reduced mosquito emergence; however, *P. reticulata* developed greater population densities than did the other species and provided mosquito control that was comparatively better than the other species.

Both native and exotic fish have been used to control mosquito populations inhabiting wells, water storage vessels and water conveyance systems. Catfish (*Clarias fuscus* (Lacepède)) in China (Wu et al. 1987), minnows [*Zacco platypus* (Temmick & Schlegel)] in Korea (Yu et al. 1980), tilapia [*Oreochromis spilurus spilurus* (Günther)] in northern Somalia (Goriup and der Kaay 1984), killifish (*A. dispar*) in Ethiopia and the Middle East (Fletcher et al. 1992, Homski et al. 1994), exotic guppies (*P. reticulata*) in French Polynesia (Lardeux 1992) as well as in India with native *Aplocheilus* (Menon and Rajagopalan 1978, Sharma et al. 1987, Kumar et al. 1998), rainbowfish [*Melanotaenia splendida splendida* (Peters)] in Australia (Russell et al. 2001) and several fish genera (*Aplocheilus, Betta, Carassius, Cyprinus, Macropodus* and *Oreochromis*) in Vietnam (Nam et al. 2000) have been used to control mosquitoes utilizing water storage vessels as developmental sites. Ghosh et al. (2005) found that *Clarias gariepinus* (Burchell) was the most effective larvivore of 4 species at reducing larval mosquitoes in a trench in India.

Three African cichlids, *Tilapia zilli* (Gervais), *Sarotherodon (Tilapia) mossambica* (Peters), and *S. hornorum* Trewazas, were introduced into southern California as biological controls of aquatic weeds, mosquitoes and chironomids (Legner and Pelsue 1983). The fish populations flourished in irrigation channels and drains, golf courses lakes, sewage treatment lagoons and flood control channels although intolerance of low temperatures caused significant overwintering mortality in habitats without thermal inlets (Legener and Medved 1973, Legner and Fisher 1980). *Tilapia* was one of the 13 fish species found to be effective against riceland mosquitoes (Lacey and Lacey 1990). Concurrent stocking of indigenous phytophagous fish and small larvivorous fish has been proposed as a simple, economical integrated control program for vector breeding habitats (WHO 1982).

Annual and seasonal fish such as *Nothobranchius* spp. (Cyprinodontiformes: Aplocheilidae) have been proposed for use against vector mosquitoes inhabiting temporary aquatic habitats. These fish cannot infest permanent water systems (WHO 1982) and prey on mosquito larvae in the laboratory (Vitlin and Artem'ev 1985, Ijumba and Kilama 1991) but large-scale field evaluations are lacking.

Ahmed et al. (1988) provided a simple rating system to help select the proper fish species for mosquito control (Table 6). The rating system can be tailored to particular regions and experience acquired. Questions that are not relevant to a particular situation where the fish are to be used can be eliminated. For example, the arroyo chub

(*Gila orcutti* Eigenmann and Eigenmann) is considered a viable replacement for *G. affinis* in large riverine wetlands used to purify river water before infiltration into groundwater reservoirs holding potable water supplies in southern California. Beneficial characteristics of the arroyo chub include the fact that it is native to southern California and is a species of concern in the drainages where its use is proposed; it has broad environmental preferences occurring both within fast-moving streams (velocities > 80 cm sec^{-1}) and in quiescent backwaters (Moyle 2002) where it co-occurs with *Gambusia* in the latter environment; it is tolerant of fluctuating water levels, elevated water temperatures and moderate hypoxia; it is omnivorous, feeding on insects and algae; although the arroyo chub lays eggs, it has a fractional reproductive strategy [reproducing nearly continuously between February and August (Moyle 2002)] that effectively brackets the peak activity of wetland mosquitoes with the production of juvenile fish; and it is capable of a rapid numerical response after introduction in wetland habitats. Even though the preference for immature mosquitoes, tolerance of low pH, salinity tolerance, and the density of fish required for control are unknown at this time, *G. orcutti* scores highly on Ahmed et al.'s (1988) rating system [a score of 102 with 4 questions unanswered and 1 question (26) not applicable] as a potential biological control agent for mosquitoes.

The successful implementation of biological control agents against mosquitoes depends on an in-depth understanding of the ecology of the targeted species, non-target organisms and the biological control agents to be used (Lacey and Lacey 1990, Lacey and Orr 1994). Laird (1985) recommended that cost/risk/benefit studies should be an essential part of any control program. Evaluation of larvivorous fish for mosquito-borne disease control should include an epidemiological evaluation, including assessments of the effectiveness of the biological control agent against the vector mosquito and its impact on disease prevalence, as well as appraisals of the socioeconomic and environmental implications of using particular larvivorous species (Wickramasinghe and Costa 1986). Such knowledge will facilitate understanding of the environmental factors that limit the efficacy of the biological control agent among different habitats within a particular region and among different geographic regions (WHO 1982).

Except in the simplest of confined ecosystems, biological control rarely functions as the sole method for the successful abatement of mosquitoes; therefore, an understanding of alternative methods for mosquito control (e.g., source reduction, chemical and biological control agents) and an appreciation of their integration with larvivorous fishes is required. Judicious use of larvivorous fish is required to avoid situations where native species are detrimentally affected by the addition of larvivorous fish. When used under appropriate conditions, larvivorous fish can be an important accompaniment for mosquito control programs, eliminating immature mosquitoes in some situations, and reducing pesticide usage and selection for resistance in target insect populations in other situations.

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FOODS OF *MYOTIS LUCIFUGUS* IN A MATERNITY COLONY IN CENTRAL ALASKA

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Foods of *Myotis lucifugus* at Salcha, near Fairbanks, Alaska, at a time of 24-h daylight were: small moths (71.1% by volume), spiders (16.8%), and mosquitos (1.8%).

Key words: *Myotis*, diet, maternity colony, Alaska

The little brown bat, *Myotis lucifugus*, occurs in summer over an extensive range in Alaska as indicated by scattered records north to the Yukon River (Hall, 1981; Manville and Young, 1965). This species appears to be uncommon in interior Alaska, but few data are available.

A maternity colony of little brown bats was located by the Alaska Department of Fish and Game in a quonset hut on a potato farm 4 km east of the mouth of the Salcha River (64°28′N, 146°53′W). Natural habitats in the vicinity of the colony were a typical mosaic of mixed spruce-birch forest, willow and alder shrubland, and grassy meadows bordering a river and associated sloughs and oxbows in interior Alaska. Of the land in the vicinity, <25% is cultivated and the Salcha River is ca. 1 km away at its closest point. JOW and colleagues visited this colony on 15 June 1989, observed the bats, and obtained a sample of relatively fresh guano from beneath the roost. It was not possible to determine when the feces were deposited, but only pellets judged to be fresh were used in the analysis. The owner of the property, K. Price, was of the opinion that the bats had returned to the colony recently from their hibernating quarters (location unknown) so presumably the feces were deposited during the 2nd week of June. The bats were roosting in a crack in polyurethane foam insulation at the back of the quonset hut. At least 70 bats were counted and perhaps more were present.

Myotis lucifugus generally feeds on aquatic insects with midges (Chironomidae) and caddisflies (Trichoptera) often being staples, but moths, various hoppers (Cicadellidae, Cercopidae), other flies (Diptera), and smaller beetles also are major dietary items (Anthony and Kunz, 1977; Belwood and Fenton, 1976; Buchler, 1986; Ross, 1967; Whitaker, 1972). We report foods eaten by the bats in the Salcha colony. The results are of special interest because of the limited foraging time imposed by the 24-h daylight at the time of collection of guano.

METHODS

For food habits analysis it is best to use stomach contents to avoid bias from differential digestion. However, because bats feed on insects and since insects contain chitin, which is relatively indigestible, one can derive reasonable estimates of foods consumed from fecal pellets of bats (Dickman and Huang, 1988; Kunz and Whitaker, 1983). One hundred fecal pellets were examined microscopically. Food items were identified by comparison of characteristic parts with a reference collection of whole organisms, and the volumetric percentage of each item was es-

timated visually in each pellet as outlined by Whitaker (1987).

RESULTS

Moths were the dominant food, occurring in 97% of the pellets and forming 71.1% of the total (Table 1). The second most abundant prey organisms were spiders. Spiders and other flightless taxa are eaten occasionally, but do not constitute a major portion of the food of North American insectivorous bats. Spiders comprised 16.8% of the total volume of food and were found in 36% of the pellets. We do not know where or how the spiders were taken, perhaps from leaves or webs by echolocation or gleaning.

Mosquitos are abundant in interior Alaska and were identified in 17% of the feces, and composed 1.8% of the total volume of food. Contrary to popular belief, few definite records exist of mosquitos being a major prey of North American bats. Mosquitos formed a staple in the diet of *Myotis austroriparius* in Florida (Zinn and Humphrey, 1983), again in an area where mosquitos were prominent. Mosquitos were found by Anthony and Kunz (1977) in 48 of 62 (74%) pellets of *M. lucifugus* examined from New Hampshire, but unfortunately no volumetric estimates were made to assess the relative importance of mosquitos in the diet. Fascione et al. (1991) reported mosquitos in feces of 33 of 39 bats (84.6%) from Ontario, Canada, but again no volumetric estimates were made. Other investigators (Buchler, 1976; Poole, 1932; Whitaker, 1972; Whitaker et al., 1977) reported mosquitos in lesser amounts. Mosquitos seldom form an important component of the food of bats, perhaps because of their tendency to remain close to vegetation rather than flying in the foraging areas generally occupied by bats.

Unidentified dipterans were present in 23% of the pellets and composed 3.7% of the total volume. Many of these were probably tipulids or culicids, but identification could not be confirmed. Tipulids often are a major food of *Myotis* (Whitaker, 1972) so their occurrence was not surprising. However, midges (Chironomidae) often are important (Belwood and Fenton, 1976; Whitaker, 1972), but they were only recorded once in our sample. The lack of midges and other aquatic forms probably indicates that these bats were feeding primarily in terrestrial sites. This could be an avoidance reaction to feeding in open areas in bright-light conditions as suggested by Nyholm (1965) and J. Rydell (in litt.).

TABLE 1.—*Frequency of occurrence and volumetric percentages of food items in 100 fecal pellets of the little brown bat,* Myotis lucifugus, *taken 15 June 1989 from under a roost in a quonset hut on a farm 11 km east of Salcha, Alaska.*

Food item	Frequency of occurrence	Volumetric percentage
Lepidoptera (moths)	97.0	71.1
Araneae (spiders)	36.0	16.8
Other Diptera (flies)	23.0	3.7
Culicidae (mosquitoes)	17.0	1.8
Other Coleoptera (beetles)	3.0	1.4
Carabidae (ground beetles)	2.0	1.1
Tipulidae (crane flies)	4.0	0.9
Hemerobiidae (brown lacewings)	5.0	0.8
Miridae (leaf bugs)	2.0	0.7
Unidentified insects	2.0	0.6
Hymenoptera (wasps)	4.0	0.5
Hemiptera (true bugs)	2.0	0.4
Dytiscidae (diving beetles)	1.0	0.3
Chironomidae (midges)	1.0	0.2
		100.3

No more than one spider was found in any pellet in our sample. The mosquitos were among the smallest prey eaten, but the most mosquitos in one pellet was three. The number of moths was difficult to estimate (because they are finely chewed), but it appeared that no more than two or three moths occurred in most fecal pellets.

DISCUSSION

Rydell (in litt.) analyzed two samples of fecal pellets from the northern bat, *Eptesicus nilssoni,* from northern Sweden (Lulea, 65°N), a locality similar to Salcha. He found

that small nematocerans (including Culicidae and Chironomidae) comprised 54% of the volume in one sample (n = 100 pellets), culicids constituted 28.6%, chironomids 3.6%, and other small dipterans formed 30.4% of the volume in the other sample (n = 28) collected 27 July 1988.

Rydell (1986, 1989) studied the diets of *E. nilssoni* and *Plecotus auritus* in southern Sweden also at a high latitude (57°, 45′N) where few species of bats were present, and assumed availability of insects to be similar for the two species since they fed in the same places at the same time. Large nocturnal flying insects, moths, beetles (mainly Scarabaeidae), crane flies, and caddisflies were the principal prey of both species, forming 47% of the diet of *E. nilssoni* and 57% of *P. auritus*. However, the remainder of the diets of these two species were quite different. About 47% of the diet of *E. nilssoni* was small dipterans, particularly Chironomidae, whereas these insects were seldom eaten by *P. auritus*. About 40% of the diet of *P. auritus* comprised taxa that were diurnal (blowflies, Calliphoridae), terrestrial, or were ground-dwelling or arboreal such as earwigs (Dermaptera), spiders (Araneae), and harvestmen (Opiliones). Rydell (1985, 1989) assumed these latter items were taken by gleaning by *Plecotus*.

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FIELD EVALUATION OF CDC AND MOSQUITO MAGNET® X TRAPS BAITED WITH DRY ICE, CO_2 SACHET, AND OCTENOL AGAINST MOSQUITOES

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ABSTRACT. The Centers for Disease Control and Prevention (CDC) light traps and Mosquito Magnet® X (MMX) traps baited with dry ice, octenol, and a new formulation (granular) of carbon dioxide (CO_2) were evaluated against adult mosquitoes in the field. The results showed that the MMX traps (68.6%) baited with dry ice collected more mosquitoes compared to the CDC light traps (32.4%) only. The CDC traps baited with dry ice (64%) collected significantly more mosquitoes than traps baited with CO_2 sachets (11%) or octenol (23%). The MMX traps baited with dry ice (85.5%) collected significantly more mosquitoes than traps baited with CO_2 sachets (6.5%) or octenol (9%). The CDC traps baited with the formulations of normal and slow release CO_2 sachets collected more mosquitoes than the formulation of fast release sachets. The CDC traps baited with fresh sachets and 24-h-exposed sachets collected significantly more mosquitoes than the traps baited with 48-h- and 72-h-exposed sachets.

KEY WORDS Carbon dioxide, sachet, chemical generation, traps, mosquitoes

INTRODUCTION

The additions of attractants, such as carbon dioxide (CO_2) and 1-octen-3-ol (octenol) to the Centers for Disease Control and Prevention (CDC) light traps greatly enhance trap collections and have become an essential element of trapping adult mosquitoes for population and arbovirus surveillance. Carbon dioxide has been used as mosquito attractant since the early 1920s. Newhouse et al. (1966) combined the use of CO_2 in the form of dry ice with CDC traps and increased mosquito catches by 400–500%. Traps with a cylinder could be used to release CO_2. The amount of dry ice as well as the type of containers used to hold the CO_2 directly affects the amount of CO_2 released over time (Kline 2006). In some regions dry ice is difficult to obtain and/or creates the need to buy a dry ice maker. Buying or renting compressed gas cylinders, refilling CO_2, and processing dry ice require increased labor and costs. Recently granular media CO_2 sachets have been developed by ICA Tri Nova (Forest Park, GA) and are commercially available from John W. Hock Company, Gainesville, FL. Octenol is another mosquito attractant (Takken and Kline 1989) commercially available.

Traps combined with attractants are a major part of mosquito population and arbovirus surveillance programs. The attractiveness, effectiveness, costs, transportation, handling, and storage of traps and attractants are major considerations in selecting a trapping method. This study presents the results of using the new granular formulation of CO_2, in comparison to commonly used attractants, octenol, strips, and CO_2 in the form of dry ice, using 2 different types of traps evaluated in St. John's County, Florida.

MATERIALS AND METHODS

Trap comparison studies

Three CDC light traps and 3 Mosquito Magnet® X (MMX) traps described by Kline (1999) and produced by American Biophysics Corporation (ABC, North Kingston, RI) and baited with dry ice in containers were used in the field for 2 nights. The species and number of mosquitoes caught in each trap were identified.

Bait comparison studies

The CDC light traps and the MMX traps, in combination with dry ice, granular formulation of CO_2 sachets, and octenol strips, were used in the field. Nine CDC light traps were separately placed at Guana Park and Allen's Farm, St. John's County. Three of the 9 traps used were baited with each type of attractants. Traps were placed in the field for 3 nights, and attractants were rotated each time so that they occupied all trapping locations. Three MMX traps combined and baited with different types of attractants were separately placed at Tocoi Terrace and State Road 16 West, St. John's County, for 3 nights, and each attractant was rotated to each location. Traps were placed in the field for 24 h.

CDC traps baited with sachets and dry ice studies

The test was conducted in Hastings, St. John's County, Florida, from October 7, 2003, to October 9, 2003. Six CDC light traps in

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combination with a granular media sachet of CO_2 and dry ice were used. Two traps were baited with the CO_2 sachets, 2 with dry ice in a container, and 2 traps without any bait were left unbaited as a control. The traps were set up in the field for 3 nights, and the bait used was rotated each night.

Formulation and persistence of CO_2 sachets

Three CDC light traps were combined with normal, slow, and fast releasing formulations of the granular media CO_2 sachets and placed at Tocoi Terrace, St. John's County, Florida. Traps were placed in the field for 3 nights, and the 3 formulations of sachets were rotated each night.

Persistence of a granular CO_2 sachet was evaluated using 4 CDC traps combined with different exposure times of sachets. One trap with a newly opened sachet was used for control, and 3 traps were used as treatments (1 trap with 24-h exposure, 1 trap with 48-h exposure, and another with 72-h exposure). The tests were conducted twice, and the traps were rotated each time.

Data analysis

Traps studies, bait comparison, and studies on formulation and persistence of the CO_2 sachets were conducted separately. Simple analysis of variance (ANOVA) was used to compare the numbers of mosquitoes caught by traps baited with attractants (CoStat 2004).

RESULTS

Species composition

A total of 18 species of mosquitoes (19,357 adult females) were collected over 4 months (July–October 2003) of the study with the most common species being *Culex nigripalpus* Theobald (59%), *Anopheles crucians* Wiedemann (15%), *Ochlerotatus atlanticus* Dyar and Knab (7.4%), *Oc. infirmatus* Dyar & Knab (5.4%), and *Psorophora columbiae* Dyar and Knab (9.3%).

Trap comparison studies

A total of 13 mosquito species (7,875) were collected using CDC traps and MMX traps baited with dry ice over the 2 nights. The CDC traps baited with dry ice collected 2,476 (32.4%), and MMX traps baited with dry ice collected 5,399 (68.6%) (F 1, 12 = 21.42, $P < 0.01$). There were 2 individual species collected only by CDC light traps (Table 1).

Bait comparison studies

A total of 15 species of mosquitoes were collected using CDC traps and MMX traps at Allen's Farm and Guana Park, St. John's County, Florida. The CDC traps baited with dry ice (2,607 or 64%) collected significantly more mosquitoes than traps baited with CO_2 sachets (520 or 11%) or octenol (941 or 23%) (Table 2; F 2, 15 = 169, $P < 0.001$).

A total of 13 mosquito species were collected at Tocoi Terrace and St. Road 16 West, St. John's County, Florida. The MMX traps baited with dry ice (4,755 or 85.5%) collected significantly more numbers of mosquitoes than traps baited with CO_2 sachets (311 or 6.5%) or octenol (496 or 9%) (Table 2; F 2, 13 = 141, $P < 0.001$).

Table 1. Number of mosquitoes caught by 3 CDC and 3 MMX traps baited with dry ice over 2 nights, St. John's County, Florida, October 2003.

Mosquito species	CDC light trap	MMX trap
Aedes albopictus Skuse	1	8
Anopheles crucians	1,174	734
Coquillettidia peturbans Walker	0	1
Culiseta melanura Coquillett	0	5
Culex erraticus Dyar and Knab	1	4
Cx. nigripalpus	1,159	4,147
Ochlerotatus atlanticus	15	46
Oc. infirmatus	51	146
Oc. mitchellae Dyar	3	31
Psorophora ciliate Fabricius	0	3
Ps. columbiae	70	274
Uranotaenia sapphirina Osten Sacken	1	0
Ur. lowii Theobald	1	0
Mean	190a*	415b*

* Mosquito numbers in the mean row followed by the same letter are not significantly different (F 1,12 = 21.42, $P < 0.01$).

CDC traps baited with CO_2 sachets and dry ice studies

The CDC light traps baited with CO_2 sachets or dry ice collected significantly more numbers of mosquitoes than traps without any bait (52 or 4.2%). Trap baited with CO_2 sachets (610 or 48%) collected a similar number of mosquitoes compared with the traps baited with dry ice (603 or 47.7%) (Table 3; F 2, 8 = 178, $P < 0.05$).

Released formulations and persistence of CO_2 sachets

The CDC traps baited with slow-release CO_2 sachets collected a similar number of mosquitoes as the traps baited with normal release sachets. The normal (68 or 36%) and slow (70 or 37%) release sachets collected more numbers of mosquitoes than the fast (49 or 26%) release sachets (Table 4; F 2, 7 = 30.99, $P < 0.01$).

The CDC traps baited with newly opened fresh sachets (104 or 29.5%) of the normal release packets collected the same number of mosquitoes

Table 2. Total number of mosquitoes caught by 9 CDC and 3 MMX traps baited with CO_2 sachets, dry ice, and octenol over 3 nights at St. John's County, Florida, July–August 2003.

	Allen Farm & Guana Park			Tocoi Terrace & SR 16W		
Mosquito species	CDC + sachets	CDC + dry ice	CDC + octenol	MMX + sachets	MMX + dry ice	MMX + octenol
Aedes albopictus	0	0	1	12	4	7
Anopheles crucians	121	342	197	6	278	6
Culiseta melanura	3	16	19	7	7	0
Culex erraticus	6	54	20	4	0	0
Cx. nigripalpus	301	1,186	302	134	2,807	85
Cx. quinquefasciatus	0	75	0	33	0	4
Ochlerotatus atlanticus	32	154	205	55	572	239
Oc. infirmatus	7	139	67	58	417	120
Oc. mitchellae	0	0	0	0	162	0
Oc. solicitans	5	2	0	0	0	0
Oc. taeniorhynchus	1	22	5	0	0	0
Psorophora ciliate	0	2	2	0	43	1
Ps. columbiae	42	591	123	2	417	34
Ps. ferox	0	13	0	0	45	0
Other	2	11	0	0	3	0
Mean	35b*	174a	63b	21B	317A	33B

* Mosquito numbers in the mean row followed by the same letter are not significantly different (Allen Farm & Guana Park, F 2, 15 = 169, $P < 0.001$; Tocoi Terrace & SR 16W, F 2, 13 = 141, $P < 0.001$).

as the traps baited with post-24-h-exposed sachets (104 or 29.5%). The traps baited with fresh sachets and 24-h-exposed sachets collected significantly more numbers of mosquitoes than the traps baited with 48-h-exposed (71 or 20%) and 72-h-exposed sachets (73 or 21%) (Table 4; F 3, 7 = 7.812, $P < 0.05$).

DISCUSSION

Trap comparison studies

Kline (1999) first reported a new counterflow geometry trap, now called the MMX trap. Usually the effectiveness, labor costs, and material are often the important factors in choosing a surveillance method (Kline 2006). Our results showed that the MMX traps baited with dry ice collected many more mosquitoes compared with the CDC light traps. The MMX trap is a new style of battery-operated trap that does not use a light source, but it is more expensive and has not been commercially available. The CDC traps are more economical, easy to transport, and battery operated.

Table 3. Number of mosquitoes caught by CDC light traps baited with CO_2 sachets, dry ice, and no attractant over 3 nights at Hastings, St. John's County, Florida, 2003.

Mosquito species	CDC + sachets	CDC + dry ice	CDC no attractant
Anopheles crucians	11	17	0
Culiseta melanura	11	0	1
Culex erraticus	1	0	0
Cx. nigripalpus	449	456	49
Ochlerotatus atlanticus	4	6	0
Oc. infirmatus	13	12	0
Psorophora ciliate	2	1	0
Ps. columbiae	114	111	2
Other	5	0	0
Mean	68a*	67a	6b

* Mosquito numbers in the mean row followed by the same letter are not significantly different (F 2, 8 = 178, $P < 0.05$).

Bait comparison studies

The most commonly used attractant CO_2 in the form of dry ice combined with the CDC light trap is extremely attractive to mosquitoes (Reisen et al. 2000). However, it is expensive to purchase (approximately \$10.00 per 2.5 kg block), it is difficult to regulate the amount being placed in the field because of container factors, and the rate of release can be altered because of different weather. Also, the dry ice can be difficult to process and transport and is dangerous to workers. The granular CO_2 sachet with CDC traps caught similar numbers of mosquitoes compared with octenol (Canyon and Hii 1997). Octenol strips are also convenient and can last for up to 3–4 months depending on storage and weather conditions. The CO_2 sachets and octenol strips are easy to carry and store and are safe to handle. However, the cost of CO_2 sachets (\$3–5 per sachet and lasting for a few days) is more than octenol strips (\$3–4 per strip and lasting for a few months).

Table 4. Number of mosquitoes caught by CDC traps baited by different release rates of CO_2 sachets (over 2 nights, August 20 and 28, 2003) and persistence of slow release rate of CO_2 sachets (over 6 nights, September–October 2003) at Tocoi Terrace, St. John's County, Florida.

	Release rates				Persistence (h)		
Mosquito species	Fast	Slow	Normal	Fresh	24	48	72
Anopheles crucians	3	1	0	26	19	22	5
Culiseta melanura	3	2	0	11	8	4	2
Culex erraticus	0	0	0	2	1	1	9
Cx. nigripalpus	6	19	29	59	72	40	55
Ochlerotatus atlanticus	33	40	29	1	0	0	0
Oc. infirmatus	0	7	9	2	1	1	0
Psorophora columbiae	4	1	1	3	2	2	1
Other	0	0	0	0	1	1	1
Mean	6A*	9B	9B	13a	13a	9b	9b

* Mosquito numbers in the mean row followed by the same letter are not significantly different (release rates, F 2, 7 =30.99, $P < 0.01$; persistence, F 3, 7 = 7.812, $P < 0.05$).

CDC traps baited with CO_2 sachets and dry ice studies

The CDC traps baited with attractants (sachets or dry ice) collected more mosquitoes than the traps without any attractant. The results are similar to reports by Kline et al. (2006) and Newhouse et al. (1966). However, the traps with dry ice and CO_2 sachets collected similar numbers of mosquitoes in this study. The results differed from data obtained from other tests. This discrepancy needs to be addressed in the future.

Released formulations and persistence of CO_2 sachets

Kline et al. (2006) reported that the sachet at 48 h was still attracting 40.5% of *Aedes aegypti* (L.) in an olfactometer in the laboratory. The results of the persistence test of sachet showed that the release formulations affected the numbers of mosquito collected. The fast release formulation collected fewer mosquitoes than the normal and slow formulations. The freshly opened or at 24-h-exposed normal sachets collected more numbers of mosquitoes than the sachets exposed for 48 h and 72 h in the field. This means the freshly opened sachet should be used during mosquito population surveillance or collection when using sachets for attractant. The granular CO_2 sachets could be used for mosquito and arbovirus surveillance under certain circumstances (Webb and Russell 2004) and are convenient for transporting, handling, and storing and are safe for workers. However, further research and field evaluation about effectiveness, released formulation, persistence, and cost are still needed.

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Cost-effectiveness of West Nile Virus Vaccination

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West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 in New York City. From 1999 through 2004, >16,600 cases of WNV-related illnesses were reported in the United States, of which >7,000 were neuroinvasive disease and >600 were fatal. Several approaches are under way to develop a human vaccine. Through simulations and sensitivity analysis that incorporated uncertainties regarding future transmission patterns of WNV and costs of health outcomes, we estimated that the range of values for the cost per case of WNV illness prevented by vaccination was US $20,000–$59,000 (mean $36,000). Cost-effectiveness was most sensitive to changes in the risk for infection, probability of symptomatic illness, and vaccination cost. Analysis indicated that universal vaccination against WNV disease would be unlikely to result in societal monetary savings unless disease incidence increases substantially over what has been seen in the past 6 years.

West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 during an outbreak of encephalitis in New York City (*1*). Over the next 6 years the virus spread across the continental United States, as well as into Canada, Latin America, and the Caribbean islands (*2*,*3*). From 1999 through 2004, >16,600 WNV illnesses in humans have been reported in the United States; >7,000 of these were neuroinvasive disease, and >600 were fatal. In 2002 alone, 2,942 cases of neuroinvasive WNV disease were reported in the United States, which represents the largest epidemic of neuroinvasive WNV disease ever recorded (*4*). Approximately 20% of WNV infections in humans result in symptomatic illness, and ≈1% of infections lead to encephalitis, meningitis, or acute flaccid paralysis (*1*). A substantial proportion of persons in whom severe neuroinvasive WNV disease develops have long-term disability or die as a result of their infection (*5*,*6*).

WNV is transmitted to humans primarily through the bite of infected mosquitoes, but transmission through blood transfusion, through organ donation, and from mother to child have been described (*7*). Strategies to prevent WNV infection include avoiding exposure to infected mosquitoes, reducing the abundance of mosquito vectors, and screening infected blood donations before transfusion. Several approaches are under way to develop a safe and effective human vaccine (*8*–*10*). The public health utility of a new vaccine will depend largely on the incidence, geographic distribution, and severity of WNV disease in the United States, as well as the cost of vaccination. We evaluated the cost-effectiveness of vaccination against WNV in the United States from a societal perspective. Uncertainties regarding the future transmission patterns of WNV and the costs of health outcomes preclude an exact estimation of the economic impact of vaccination. Through probabilistic sensitivity analysis, which incorporates these uncertainties, we estimated the range of most likely values for the cost-effectiveness of vaccination and described the variables that have the most impact on the economic outcome of vaccination. We also estimated the likelihood that a universal vaccination program would result in economic savings.

Methods

The decision tree used to evaluate the cost-effectiveness of vaccination compared with no vaccination is shown in the Figure. Vaccination was assumed to have no effect on the incidence of infection or the severity of WNV illness but rather to influence only the proportion of infected persons in whom symptoms would develop. Baseline probabilities for each of the chance nodes in the tree were derived by reviewing published articles on the incidence, clinical manifestations, and outcomes of WNV disease as described in further detail below. We estimated the average cost per case of WNV illness prevented, that is, average cost-effectiveness ratio (ACER) (online Appendix 1, available from http://www.cdc.gov/ncidod/EID/vol12no03/05-0782_app1.htm) by calculating the expected societal costs of WNV illness with a vaccination strategy, subtracting the costs of illness with no vaccination, and dividing the

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Figure. Decision tree for vaccination program. WNV, West Nile virus.

remainder by the number of cases prevented by vaccination.

Time Horizon

We assumed that a single dose of live-attenuated WNV vaccine would provide immunity for ≥10 years, as is true for the currently licensed yellow fever vaccine (*10,11*). If an inactivated vaccine were used, 2 or 3 initial doses would probably be required, and booster doses would probably be needed every 3 years, as is currently recommended for inactivated Japanese encephalitis vaccine (*12*). Both the cost and effectiveness of vaccination were assumed to be the same whether achieved through a single live-attenuated vaccine dose or multiple inactivated vaccine doses at a lower cost per dose.

Although the time horizon for risk for illness, protection from the vaccine, and cost of vaccination was 10 years, we used estimated lifetime costs of disease outcomes in our model. Thus, we modeled the difference in lifetime costs of illness that would be incurred by society during a 10-year period under an immediately implemented universal vaccination strategy compared with no vaccination.

The probabilities of outcomes and costs modeled are average probabilities for the entire population, regardless of age. Our analysis therefore estimates ranges of average societal costs and outcomes prevented when all people in the society are vaccinated, regardless of the age at vaccination or illness. A more detailed analysis of the effect of vaccinating certain age groups would require estimates of age-specific risks and costs of outcomes, which are not readily available for most outcomes in the model.

Estimation of Costs

The overall cost of WNV illness per person at risk was calculated as the sum of the average costs for each health outcome weighted by the probability of occurrence of each outcome (online Appendix Table, available from http://www.cdc.gov/ncidod/EID/vol12no03/05-0782.htm#table_app). Both medical treatment costs and productivity losses due to illness and death from WNV infection were included in cost estimates. We considered the following health outcomes of WNV infection in our analysis: asymptomatic infection, uncomplicated febrile illness with full recovery, neuroinvasive illness (encephalitis, meningitis, or paralysis) with full recovery, neuroinvasive illness with residual long-term disability, and death.

Asymptomatic infection was assumed to have no cost. Estimates of the cost of uncomplicated febrile illness due to WNV infection were not available so we assumed a cost of US $1,000 per case, based on 5 days of lost productivity at $165 per day (*13*), plus an assumed $175 in medical costs that included 1 ambulatory care visit, diagnostic tests, and outpatient medications. Precision of this cost estimate was not very important since the cost-effectiveness ratio was not sensitive to the changes in this variable. The estimated cost per case of neuroinvasive WNV illness with full recovery ($27,500) was derived from an economic study conducted during the 2002 WNV epidemic in Louisiana (*14*) in which economic costs, rather than charges, were considered a measure of resources. Our goal was to measure the forgone benefits that could have been derived if the resources had been allocated to their next best use, i.e., the opportunity cost. Charges made by healthcare providers do not usually reflect the opportunity costs because of healthcare market imperfections. Charges were adjusted to economic costs through the use of cost-to-charge ratios (for details see Appendix 2 in reference *14*; we adjusted 2002 dollars to 2004 dollars, the last year for which consumer price indices were available at the time of this study [*15,16*]). This cost of neuroinvasive illness included costs of outpatient evaluation, inpatient treatment, rehabilitation treatment, lost productivity of the patient and caregiver at home, and transportation (online Appendix Table). Estimates of the cost of residual long-term disability after neuroinvasive disease were not available, but many of the disabilities that have been described after WNV illness are clinically similar to those that result from acute stroke, and the 2 conditions both affect primarily older males. We therefore used estimates of the lifetime cost of stroke as a proxy for the cost per case of neuroinvasive WNV illness with residual long-term disability (*17*) (1990 dollars adjusted to 2004 dollars [*15,16*]). Details are shown in online Appendix 2 (available from http://www.cdc.gov/ncidod/EID/vol12no03/05-0782_app2.htm).

The average societal cost due to death from WNV disease was estimated by using productivity loss tables (*13*) and the age distribution of 713 WNV nationwide deaths reported to the ArboNET database of the Centers for Disease Control and Prevention (CDC) since 1999 (CDC, unpub. data). The median age of fatal cases was 77 years (range 1 month to 99 years). The estimated cost due to death was $200,000 at a 3% discount rate (2000 dollars from productivity tables [*13*] were adjusted to 2004 dollars [*16*]). Since short-term costs in our model were randomly distributed throughout the 10-year time horizon, to simplify the model, we only discounted the long-term costs, such as long-term disability costs and costs due to death. For the short-term costs incurred within the 10-year time horizon, we assumed our estimates represented the present values of those costs (online Appendix 2, available from http://www.cdc.gov/ncidod/EID/Vol12No03/05-0782_ app2.htm).

Since no human WNV vaccine was licensed at the time of our evaluation, vaccine costs were not available. Based on charges in the United States for yellow fever vaccine (≈$85 per dose), hepatitis A vaccine (≈$75 per dose), Japanese encephalitis vaccine (≈$315 for a 3-dose series), and the previously available Lyme disease vaccine (≈$150 for a 3-dose series), we assumed a total baseline vaccination cost of $100 to include both the actual cost of the vaccine and the cost of administering the vaccine. For the sensitivity analysis focused on the cost of vaccination, we assumed minimum and maximum vaccination costs of $10 and $150, respectively (see below).

Estimates of Probabilities for Health Outcomes

Probability of Infection

Several seroepidemiologic surveys have estimated the proportion of North American populations who were infected with WNV during epidemic transmission. The highest seroprevalence published to date is 2.6% (*1*). In 2002, during the largest epidemic of WNV neuroinvasive disease ever described in the United States, 2,942 neuroinvasive WNV disease cases were reported from 36 states and the District of Columbia (total population ≈253.4 million). If one assumes a ratio of 1 neuroinvasive case for every 140 infections, which was the finding of a 1999 household-based seroepidemiologic survey in New York City (*1*), this yields an overall estimate of ≈411,880 infections and an estimated incidence of 0.16 infections per 100 people, or 0.0016 per person per year. Whether WNV epidemics will continue to occur in the United States at a similar frequency or intensity is unknown, but for this analysis we assumed that the risk for WNV infection would be 0.0016 per person per year for 10 years. The cumulative risk for WNV infection over a 10-year period would be $1 - e^{(-0.0016 \times 10)} = 0.016$. We therefore estimated the baseline probability of infection as 0.016. For sensitivity analysis focused on probability of infection, we assumed for the minimum risk for infection that a person would encounter only 1 year of WNV transmission, yielding a cumulative risk of 0.0016 over the 10-year period. For the maximum risk, we assumed that the risk would be that of yearly epidemic transmission such that 2.6% of the population would be infected each year over the 10-year period, yielding a 10-year cumulative risk of 0.23. Further details regarding sensitivity analysis are described below.

Probability of Symptomatic Illness and Vaccine Effectiveness

We assumed that symptoms of WNV illness will develop in 20% of infected persons and that neuroinvasive disease will develop in 3.6% of them, which is equivalent to 1 neuroinvasive case for every 140 infections previously described (*1*). We also assumed a vaccine effectiveness of 80% in reducing the risk for symptomatic illness.

Probability of Long-term Disability or Death after Neuroinvasive WNV Disease

Precise data on long-term outcomes from WNV illness are limited. A study of 19 patients with neuroinvasive WNV disease found that 2 (11%) died, and of the 17 survivors, 7 (41%) had recovered fully at the time of discharge, 6 (31%) were discharged without full recovery, and 4 (24%) were discharged to a long-term care facility (*18*). Another study of 57 patients with neuroinvasive disease found that 10 (18%) eventually died, 13 (23%) were discharged without support, 14 (25%) were discharged requiring support, 14 (25%) were discharged to a rehabilitation facility or nursing home, 4 (7%) moved in with relatives, and 2 (4%) remained in an acute care facility (*5*). A study of 16 patients with neuroinvasive WNV disease found that 1 patient (6%) died and that 8 months after illness, 4 (25%) patients required assistance or rehabilitation and 11 (69%) were functioning independently at home (*19*). A survey of 35 patients who had been hospitalized with WNV illness found that 63% reported full recovery 12 months after illness onset (*6*). Based on the limited data from these studies, we assumed that 35% of patients would have lifelong disability after neuroinvasive WNV disease. Of 2,942 patients with neuroinvasive WNV disease reported in the United States in 2002, 276 (9%) died (*4*). For our model, we assumed a case-fatality ratio of 9%.

Sensitivity Analysis

To incorporate uncertainties regarding the values of all input variables, we assigned uniform probability distribution to all variables, allowing 25% variability around the baseline values (Table 1). We used @Risk Analysis 2002 software (Palisade Corporation, Newfield, NY, USA) to

generate distributions of possible outcomes by Monte Carlo simulation of the ACER using 5,000 iterations that covered all combinations of input variable values. The results provided detailed summary statistics for the ACER distribution, including the 5th and 95th percentile ranges of values and the probability that vaccination would result in societal savings. To further investigate the impact of the risk for infection and vaccination cost on the ACER, we ran separate simulations in which the minimum, baseline, and maximum values for these variables described in the corresponding sections were fixed, while all other variables were allowed to vary according to their prespecified uniform distributions.

Results

Using baseline values of all input variables, without accounting for uncertainties, the average cost per case of WNV illness prevented would be ≈$34,200. At a cost of $8.7 billion in a hypothetical population of 100 million people, vaccination would prevent 256,000 cases of WNV illness, including 9,216 cases of neuroinvasive disease, 2,935 cases of lifetime disability, and 829 deaths during a 10-year period. Under these assumptions, universal vaccination would yield societal savings if the cumulative incidence of WNV infection over a 10-year period were ≥0.13 (≈1.4% of the population infected each year), the cost of vaccination were ≤$12.8, or the cost of lifelong disability were ≥$3.2 million (≈15 times higher than the baseline estimate).

The simulation results accounting for uncertainties in all input variables are shown in Table 2. The median of the ACER distribution was $35,000 per case of WNV illness prevented. The 5th and 95th percentiles for the ACER were $59,000 and $20,000, respectively.

To identify the sensitivity of the output to all input distributions, we used @Risk sensitivity analysis with a regression in which the dependent variable was the output variable, i.e., ACER, and the independent variables were the input variables presented as @Risk uniform distribution functions (Table 1). Each iteration represented an observation for the regression. The coefficients calculated for each input variable measured the sensitivity of the output to that particular input distribution. The results indicated that ACER was most sensitive to the changes in the risk for infection, probability of symptomatic illness, and vaccination cost (Table 3). A 1 standard deviation (SD) increase in the probability of symptomatic illness increased the ACER by an SD of 0.65, while a 1 SD increase in the probability of infection or the vaccination cost increased the ACER by an SD of 0.5. Changes in the other variables had little or no impact on ACER (Table 3).

The results of the sensitivity analysis focused separately on risk for infection and vaccination cost are shown in Table 4. The probability that vaccination would yield societal savings changed from 0% to 98% when the 10-year cumulative risk for WNV infection changed from 0.016 to 0.230, and from 0% to 76% as the vaccination cost decreased from $150 to $10.

Discussion

The economic impact of a vaccination strategy is a determinant of the public health decision regarding whether or not to recommend vaccination, but it is certainly not the only determinant of sound public health vaccination policy. It is also not imperative that a vaccination program result in monetary savings for it to be cost-effective compared with other public health interventions. Societies and people are willing to pay for preventing disease, as indicated by the implementation of preventive interventions that do not result in economic savings, and most relevant, the willingness to pay for expensive vaccines (*20–22*). However, as public health implications of vaccination programs are considered, we must have some understanding of the resources that might be expended. Vaccination would be most appealing if it is likely to safely prevent disease and save society money, or at least have a relatively low cost per case of illness prevented.

Table 1. Uniform distributions for each variable used in simulations to assess the cost-effectiveness of vaccination against West Nile virus (WNV)*

Variable	Lower limit	Baseline	Upper limit
Probability of infection	0.012	0.016	0.02
Probability of symptomatic illness	0.15	0.20	0.25
Probability of symptomatic illness after vaccination†	0.03	0.04	0.05
Probability of neuroinvasive disease, given symptoms	0.027	0.036	0.045
Probability of death, given neuroinvasive disease	0.07	0.09	0.11
Probability of disability, given neuroinvasive disease	0.26	0.35	0.44
Cost of neuroinvasive disease	$20,625	$27,500	$34,375
Cost of death (direct and indirect financial losses)	$150,000	$200,000	$250,000
Cost of lifelong disability	$158,000	$210,000	$263,000
Cost of uncomplicated WNV febrile illness	$750	$1,000	$1,250
Cost of vaccination	$75	$100	$125

*Upper and lower limits are calculated as ±25% of the baseline values and rounded up.
†Baseline vaccine effectiveness is assumed to be 80%.

Table 2. Outcome distributions of average cost-effectiveness ratio (ACER) accounting for variability in all input variables*

Statistic	ACER†
5th–95th percentile range, $	–59,000 to –20,000
Mean, $	–36,000
Median, $	–35,000
Mode, $	–33,000
Probability of savings, %	0

*According to the distribution provided in Table 1.
†Negative value indicates cost.

Our analysis indicates that a universal vaccination program to prevent WNV disease would be unlikely to result in societal monetary savings unless the incidence of the disease increases substantially over what has been seen in the past 6 years, or the cost of vaccination were <$12 per person vaccinated. The risk for WNV infection, probability of symptomatic illness after infection, and cost of vaccine appeared to have the greatest influence on the cost-effectiveness outcome. Within the range of possible values used in our model, variations in vaccine effectiveness, cost of WNV illness, and probabilities of various health outcomes did not lead to considerable change in the cost-effectiveness.

The future patterns of WNV transmission in North America cannot be accurately predicted. The virus was first detected in North America in 1999, and the epidemiology of WNV illness in the Western Hemisphere continues to evolve. The antigenically related flaviviruses St. Louis encephalitis virus (SLEV) and Japanese encephalitis virus (JEV) demonstrate different patterns of transmission that WNV could assume; SLEV is sporadically transmitted in North America with intense epidemics separated by years of low-level transmission, while JEV occurs in Asia with annual epidemics of intense transmission. If WNV assumes a transmission pattern in North America similar to that of JEV in Asia, then vaccination is likely to be a much more appealing public health prevention strategy and is likely to be more cost-effective than if WNV transmission follows the pattern of SLEV. As WNV spreads southward into Latin America, increased incidence may be seen with less protection from mosquitoes provided by air conditioning and screens (*23*). If intense transmission is seen in these areas, vaccination may be the most cost-effective prevention strategy, but unless the vaccine cost is low, it may still be too expensive for local economies.

WNV infection may cause severe untreatable neurologic disease. While the risk is highest in the elderly, severe disease does occur among young adults and children (*4*,*24*). The more severe, untreatable manifestations of WNV infection would compel interest in vaccine development and use even if vaccination is expensive, particularly since current prevention strategies such as personal repellent use or environmental reduction of mosquito abundance may not be consistently implemented (*25*). The effectiveness of these other prevention strategies is difficult to conclusively demonstrate and estimates of their cost-effectiveness have not been published. Vaccination may reduce the expenditures for mosquito control in certain areas, but we did not include this possible effect in our model. If alternate prevention costs were reduced by vaccination, we would expect this to improve the cost-effectiveness of vaccination from the societal perspective.

Our results provide a general assessment of the likely economic implications of universal vaccination against WNV and an indication of which parameters have the greatest influence on the cost-effectiveness of vaccination. A safe and effective vaccine may prove to be the most effective, and perhaps the most cost-effective, strategy to prevent severe WNV illness. The economic impact of vaccination will depend mostly on the risk for WNV infection, probability of symptomatic illness after infection, and the cost of vaccination.

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Table 3. Sensitivity of the average cost-effectiveness ratio (ACER) for input variables

Rank	Input variables	Regression coefficient†
1	Probability of symptomatic illness	0.65
2	Probability of infection	0.51
3	Vaccination cost	0.50
4	Probability of symptomatic illness after vaccination	–0.14
5	Probability of neuroinvasive disease, given symptoms	0.05
6	Cost of lifelong disability	–0.03
7	Probability of disability, given neuroinvasive disease	0.03
8	Cost of neuroinvasive disease	–0.02
9	Cost of uncomplicated WNV febrile illness*	–0.01
10	Cost of death (direct and indirect financial losses)	–0.01
11	Probability of death, given neuroinvasive disease	0.00

*WNV, West Nile virus.
†@Risk analysis software runs a regression where the dependent variable is the output variable, i.e., ACER, and the independent variables are the input variables presented as @Risk uniform distribution functions. Each iteration represents an observation for the regression. The coefficient calculated for each input variable measures the sensitivity of the output to that particular input distribution. For example, a coefficient of 0.65 indicates that a 1–standard deviation (SD) increase in probability of symptomatic illness increases the ACER by an SD of 0.65.

Table 4. Sensitivity of the average cost-effectiveness ratio for stepwise changes in infection rate and vaccination cost*

	Infection rate			Vaccination cost		
Statistic	0.0016	0.016	0.23	–150	–100	–10
5th–95th percentile range, $	–585,000 to –261,000	–54,000 to –22,000	343 to 3,846	–86,000 to –36,000	–56,000 to –23,000	–1,400 to 2,900
Mean, $	–400,000	–36,000	2,096	–57,000	–36,000	860
Median, $	–386,000	–34,000	2,098	–55,000	–35,000	920
Mode, $	–373,000	–30,000	1,500	–54,000	–36,000	740
Probability of savings, %	0	0	98	0	0	76

*All other variables were allowed to vary according to their specified uniform distributions provided in Table 1. Negative value indicates cost; positive value indicates monetary saving.

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ACUTE TOXICITY OF RESMETHRIN, MALATHION AND METHOPRENE TO LARVAL AND JUVENILE AMERICAN LOBSTERS (*HOMARUS AMERICANUS*) AND ANALYSIS OF PESTICIDE LEVELS IN SURFACE WATERS AFTER SCOURGE™, ANVIL™ AND ALTOSID™ APPLICATION

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ABSTRACT Acute toxicity and immune response, combined with temperature stress effects, were evaluated in larval and juvenile American lobsters (*Homarus americanus*) exposed to malathion, resmethrin and methoprene. These pesticides were used to control West Nile virus in New York in 1999, the same year the American lobster population collapsed in western Long Island Sound (LIS). Whereas the suite of pesticides used for mosquito control changed in subsequent years, a field study was also conducted to determine pesticide concentrations in surface waters on Long Island and in LIS after operational applications. The commercial formulations used in 2002 and 2003—Scourge, Anvil and Altosid—contain the active ingredients resmethrin, sumithrin and methoprene, respectively. Concentrations of the synergist piperonyl butoxide (PBO) were also measured as a proxy for pesticide exposure. Acute mortality in Stage I-II larval lobsters demonstrated that they are extremely sensitive to continuous resmethrin exposure. Resmethrin LC50s for larval lobsters determined under flow-through conditions varied from 0.26–0.95 $\mu g\ L^{-1}$ in 48- and 96-h experiments at 16°C, respectively. Increased temperature (24°C) did not significantly alter resmethrin toxicity. Malathion and methoprene were less toxic than resmethrin. The 48-h LC50 for malathion was 3.7 $\mu g\ L^{-1}$ and methoprene showed no toxicity at the highest (10 $\mu g\ L^{-1}$) concentration tested. Phenoloxidase activity was used as a measure of immune response for juvenile lobsters exposed to sublethal pesticide concentrations. In continuous exposures to sublethal doses of resmethrin (0.03 $\mu g\ L^{-1}$) or malathion (1 $\mu g\ L^{-1}$) for 7 d at 16 or 22°C, temperature had a significant effect on phenoloxidase activity ($P \leq 0.006$) whereas pesticide exposure did not ($P = 0.880$). The analytical methods developed using high performance liquid chromatography coupled to time-of-flight mass spectroscopy (LC-TOF-MS) provided high sensitivity with mass detection limits of 0.1–0.3 ng L^{-1}. Pesticide levels were often detected in the ng L^{-1} range in Long Island surface waters and western LIS (except in open waters), but rarely at concentrations found to be toxic in flow-through laboratory exposures, even immediately after spray events.

KEY WORDS: *Homarus americanus*, American lobster, resmethrin, malathion, methoprene, toxicity, immune response, LC-TOF-MS

INTRODUCTION

The spread of West Nile virus focused attention on the human and ecologic risks associated with using chemical insecticides to manage the virus's mosquito vectors. Common synthetic insecticides used for this purpose are organophosphates that function by inhibiting the nervous system enzyme acetylcholinesterase (Kennedy 1991), type I pyrethroid adulticides that modulate nervous system sodium channels (Bradbury & Coats 1989), and methoprene-based larvicide formulations that function as insect growth regulators (Celestial & McKenney Jr. 1994). The organophosphate malathion, and pyrethroids sumithrin and resmethrin, were used extensively in New York City during September 1999, immediately after the first outbreak of West Nile virus in the United States. Concurrent with the outbreak, the American lobster (*Homarus americanus* H. Milne Edwards, 1837) population in Long Island Sound (LIS) began showing signs of a massive die-off that was most pronounced in western LIS, closest to the region of spraying (CTDEP 1999). Contributing to the concern about the juxtaposition of pesticide spraying and lobster mortality was the occurrence of tropical storm Floyd that delivered more than eight inches of rain to the New York metropolitan area on September 16, 1999, subsequent to a period of intensive pesticide spraying (K. Chytalo, pers. comm.). Results of attempts to determine the exact cause of lobster mortality in 1999 were inconclusive, with the high incidence of a *Paramoeba* sp. infection in the nervous tissue of dead and dying lobsters (Russell et al. 2000) the only significant finding. This allowed for the possibility that pesticide exposure combined with other stressors (e.g., hypoxic bottom waters, elevated temperatures) may have contributed to the death of the lobsters or caused them to be more susceptible to opportunistic diseases.

Pyrethroids are highly toxic to fish with 96-h LC50s ranging from <1 $\mu g\ L^{-1}$ to 10 $\mu g\ L^{-1}$ in flow-through exposures (reviewed in Bradbury & Coats 1989 and Coats et al. 1989). Estuarine and marine crustaceans are generally more sensitive with 96-h LC50s reduced by as much as two orders of magnitude (Cripe 1994). Compared with other pyrethroids, resmethrin and structurally similar sumithrin have received little attention. Rand (2002) reviewed available data on resmethrin, most of it from unpublished reports, citing 48- to 96-h LC50s for aquatic invertebrates and fish that ranged from 0.22–15 $\mu g\ L^{-1}$. Malathion, by comparison, ranked among the least toxic of three organophosphate and three pyrethroid insecticides to the mysid shrimp *Mysidopsis bahia*, with a flow-through 96-h LC50 value of 2.6 $\mu g\ L^{-1}$ (Cripe 1994). De Guise et al. (2004) recently reported a 96-h LC50 of 38 $\mu g\ L^{-1}$ for adult *H. americanus* exposed to malathion in static tests. Methoprene has the lowest reported acute toxicity of the pesticides used to mitigate West Nile virus. *Mysidopsis bahia* exposed continuously to the more potent isomer, *S*-methoprene, showed no mortality at 62 $\mu g\ L^{-1}$ and complete mortality at 125 $\mu g\ L^{-1}$ in 96-h flow-through experiments (McKenney Jr. & Celestial 1996). Sublethal effects of these pesticides, including immunosuppression, growth inhibition, developmental delays and reproductive effects, have been observed at exposure concentrations significantly less

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than those that cause mortality (McKenney Jr. & Celestial 1996, Rand 2002, De Guise et al. 2004).

One measure of immune response is phenoloxidase (PO) activity. Phenoloxidase is an important enzyme in crustacean defense and recognition that mediates the melanization reaction around foreign substances and plays a role in wound healing, cuticle pigmentation, and sclerotization (Söderhäll 1982). Several studies have reported decreased PO activity in crustaceans exposed to environmental toxicants such as PCBs (Smith & Johnston 1992), copper sulfate (Cheng & Wang 2001), harbor dredge spoils (Smith et al. 1995), and benzalkonium chloride (Cheng et al. 2003).

For pesticides such as pyrethroids that are of toxicologic concern at concentrations in the $\mu g\ L^{-1}$ range, standard analyses need to be modified to allow detection at low ng L^{-1} levels. Analytical methods with detection limits exceeding this are inadequate for assessing the occurrence, risk or environmental fate of such compounds in surface waters. Pyrethroids have rarely been detected in surface waters after spray events because of low application rates, insensitive analytical methods and possible removal by environmental processes such as photochemical transformation and sediment sorption. Recently, Weston et al. (2004) showed that various synthetic pyrethroids can be detected in surface sediments of streams carrying pesticide run-off and, in several cases, the reported levels were of toxicologic concern.

The objectives of this study were to assess acute toxicity and immune suppression in larval and juvenile *H. americanus* exposed to malathion, resmethrin and methoprene, the pesticides used most commonly in New York to control West Nile virus, and to determine effects of temperature stress. A field campaign was also conducted to measure pesticide levels in surface waters on Long Island and in LIS after operational application of pesticides in 2002 and 2003. The analysis used methods providing detection limits at ng L^{-1} levels and below, and provides an initial data set allowing comparisons of observed surface water pesticide concentrations to those shown to harm larval and juvenile lobsters. These data should be useful in future assessments of the ecologic risks associated with pesticide application for mosquito control in coastal areas.

MATERIALS AND METHODS

Chemicals

Resmethrin (98% pure, a mixture containing 90% trans and 10% cis isomers), malathion (98% pure), and methoprene (98% pure, racemic mixture of R and S isomers) were obtained from Chem Service, Inc. (West Chester, Pennsylvania). Deuterated *d*-6 malathion was from CDN Isotopes (Quebec, Canada) and terbuthylazine was from Crescent Chemical (Islandia, New York). All solvents were analytical grade Burdick and Jackson (VWR Scientific Products, Bridgeport, New York). All other chemicals were from Sigma Aldrich (St. Louis, Missouri).

Experimental Animals and Dosing System

Larval and juvenile lobsters were obtained from the Lobster Rearing and Research Facility at the New England Aquarium in Boston, Massachusetts. For the larval experiments, 1- to 2-day-old animals were shipped overnight to our laboratory and acclimated upon arrival for 2–6 h to 16°C or 24°C, respectively, prior to pesticide exposure. The short acclimation time ensured the lobsters did not molt into stage III larvae prior to the conclusion of the experiments. Compared with earlier larval stages, stage III larvae are increasingly cannibalistic (D. Fiore, pers. comm.), which would have contributed to control morality. Most of the larvae remained at stage I throughout the exposure period based on microscopic analysis. Larvae were fed as described by Goldstein and Bartko (1999) using a 1:1 wet weight mixture of live brine shrimp nauplii (Gold Label, Argent Laboratory, Redmond, Washington) and frozen adult brine shrimp (Kordon, Royal Pet Supplies, Brentwood, New York). Experiments with juvenile lobsters were conducted with animals ranging in age from 1- to >2-y old (2.4 g to 21.2 g wet weight and 4.5 cm to 9.3 cm total length). These animals were acclimated for 7 days to 16°C or 22°C prior to pesticide exposure, and given small PVC tubes to serve as surrogate burrows. Juveniles were fed frozen adult brine shrimp every 3rd day (Goldstein & Bartko 1999).

All animals were housed in 2-L beakers and kept on a 10-h light:14-h dark photoperiod in a flow-through system at a salinity of 27 ppt. Seawater was delivered at a flow rate of 500 mL min^{-1} to the larvae and 200 mL min^{-1} to the juvenile lobsters. The experimental system consisted of a head tank delivering water by constant gravity flow to a system of 6 diluter boxes. Each box received a carrier solvent (ethanol or acetone) or a pesticide dosing solution delivered by a multichannel WPI model sp220i syringe pump (World Precision Instruments, Sarasota, Florida) at a flow rate of 3 $\mu L\ min^{-1}$. The contents of each diluter box were routed to 4 or 5 replicate chambers, and the waste effluent passed through a charcoal trap before exiting the system. Either 20 larvae or a single juvenile lobster was placed in each beaker. At the end of the exposure period, larvae were sieved from the beaker, rinsed into Petri plates, and the survivors enumerated. Larvae were considered dead when there was no visible heartbeat and gentle prodding produced no movement. Surviving juveniles were removed from the chambers and kept in a frozen seawater slurry as an anesthetic until hemolymph was drawn to assay phenoloxidase activity. The molt stage was determined from the pleopods of each juvenile at the end of the exposure period (Aiken 1991).

Measured levels of the most soluble pesticide tested, malathion, in exposure chambers were found to be 98% ± 28 SD of nominal concentrations and demonstrated that the system was effective at supplying a known and constant dose of pesticide in a flow-through mode. Measured levels of the much more particle reactive resmethrin and methoprene were inconsistent and generally higher than nominal levels most certainly because of inadvertent sampling of fine detrital particles that accumulated in the chambers. Because the exposures to dissolved pesticides were fixed by the relative flows from the syringe pump and seawater reservoir, the aqueous exposure levels are reported here, based on nominal concentrations.

Larval Experiments

Most of the work focused on resmethrin. Lobster larvae were exposed to nominal concentrations of resmethrin ranging from 0.03–0.80 $\mu g\ L^{-1}$ in a flow-through system for 48–96 h. Exposures were conducted at stressful (24°C) and nonstressful (16°C) temperatures. Larval lobsters were exposed to nominal concentrations of malathion ranging from 0.62–50 $\mu g\ L^{-1}$ in exposures conducted for 48 h at 16°C. Similar experiments were conducted with methoprene at concentrations ranging from 0.12–10 $\mu g\ L^{-1}$ for 48 h at 16°C.

Juvenile Experiments

Juvenile lobsters were exposed continuously for 7 d in a flow-through system to either resmethrin or malathion at sublethal concentrations. Initial experiments with resmethrin indicated some organisms at the highest dose tested (0.10 μg L^{-1}) showed severe locomotor impairment (animals lost ability to maintain normal posture ending up on their backs, waving their legs), therefore sublethal effects were evaluated at a lower concentration, approximately 25% of the LC50s determined from the larval exposures (0.03 μg $L^{-1}$and 1.0 μg L^{-1} for resmethrin and malathion, respectively). Juvenile lobsters acclimated to 24°C showed significant signs of stress (reduced pigmentation and lack of responsiveness), therefore 22°C was chosen as a stressful, but nonlethal temperature, with 16°C again used as a nonstressful temperature. Hemocyte PO activity was measured at the end of the 7-d exposure to assess the immune response.

Phenoloxidase Assay

Methods to analyze PO activity in lobster hemocytes were adapted from Söderhäll and Smith (1983) and Hernández-López et al. (1996). Hemolymph was drawn from the cardiac hemal sinus of juvenile lobsters using a 26-gauge needle, into a 1-mL syringe containing ice-cold citrate/EDTA buffer (0.45 M NaCl, 0.1 M glucose, 30 mM trisodium citrate, 26 mM citric acid and 10 mM EDTA at a pH of 4.6) at one or more volumes of hemolymph collected. The hemolymph was spun at 800 g (Marathon 26KMR centrifuge, Fisher Scientific, Hampton, New Hampshire) for 25 min at 4°C to pellet the hemocytes. After supernatant removal, the remaining pellet was washed twice, without resuspension, with sodium cacodylate buffer (10 mM sodium cacodylate and 10 mM $CaCl_2$ at a pH of 7.0). The pellet was resuspended in cacodylate buffer and disrupted using an ultrasonic homogenizer (4710 series, Cole-Parmer Instrument Co., Vernon Hills, Illinois). The cell suspension was centrifuged at 1200 g for 30 min at 4°C to remove the cell debris, and the hemocyte lysate supernatant (HLS) was assayed for PO activity.

A microtiter plate version of the assay was conducted by adding 50 μL of HLS to a microwell and incubating for 1 h at 25°C with either 50 μL 0.1% trypsin in cacodylate buffer (from porcine pancreas) as an elicitor, or 50 μL cacodylate buffer alone as a control. For each sample, one control and two standard reactions were run. After the incubation, 50 μL of L-dihydroxyphenylalanine (L-dopa, 3 g L^{-1} in Milli-Q water) were added as the substrate to every well. Phenoloxidase activity was determined by following the production of dopachrome from L-dopa at 490 nm with readings taken every minute for 15 min with a Wallac 1420 Multilabel Counter (Perkin Elmer, Wellesley, Massachusetts). After subtraction of control values, the rate change in absorbance of the linear portion of the curve was averaged for the two duplicate runs and normalized to the protein content.

Protein content of each hemocyte lysate was determined using the Pierce bicinchoninic acid protein assay microwell plate protocol. Bovine serum albumin was used as the standard. The 96-well plate was incubated at 25°C for 1 h and read at 560 nm on a Wallac 1420 Multilabel Counter (Perkin Elmer, Wellesley, Massachusetts).

Statistical Analyses

LC50s were determined using the Probit method (EPA Probit Analysis Program) or the Trimmed Spearman-Karber method if the assumptions for the Probit method were not met (Hamilton et al. 1977). Juvenile PO activity data were analyzed using Minitab Statistical Software (Minitab Inc., State College, Pennsylvania). Comparisons of PO response with the control values were conducted with a 1-way analysis of variance (ANOVA) and a posthoc Dunnett test. Dixon's test was applied to detect outliers (Sokal & Rohlf 1995). The effects of temperature and pesticide exposure were compared with a 2-way ANOVA. Differences were considered significant when $P < 0.05$.

Pesticide Environmental Sampling

Water samples from laboratory exposures were sampled with 10-mL volumetric glass pipets, and added to amber glass screw cap test tubes containing 1 mL of hexane and surrogate standard. For malathion analyses, samples were acidified to pH 2 with HCl to prevent base catalyzed hydrolysis during storage before analysis.

Grab samples of surface waters around Long Island were collected for pesticide analyses. During the summer of 2002, United States Geological Survey (USGS) personnel collected most of the environmental samples (United States Geological Survey). Sequential replicate samples were analyzed by the USGS laboratory in Lawrence, Kansas, and by Stony Brook University (SBU). The samples collected by the USGS included the air water interface and were collected within an hour of aerial or truck-based spraying. Samples analyzed by the USGS were further vacuum filtered through 142-mm precombusted glass fiber filters (Gelman type AE) and samples were shipped overnight on ice, unpreserved, in 1-L amber glass bottles for extraction the next day. Samples analyzed at SBU were stored in 1-L amber glass bottles with approximately 100 mL of headspace. Within 2 h of collection, 25 mL of hexane were added to each bottle and the samples were shaken. Additional samples were collected for analysis by SBU approximately 0.3 m below the water surface. All samples were stored in the refrigerator until analysis.

Pesticide spraying of coastal areas adjacent to LIS has declined significantly in New York since 1999, with no aerial spraying in Nassau County and very little along the north shore of Suffolk County. New York City, however, continues to spray, albeit on a more limited basis. Therefore, to assess potential levels of pesticides in LIS coastal waters, operational sprays in New York City and Nassau County were followed in 2003.

Pesticide Extraction and Analysis

Three methods were used to analyze aqueous concentrations of pesticides in this study. Detailed descriptions of the methods and instrumental analysis used to analyze the field samples can be found elsewhere (Zimmerman et al. 2001, Brownawell & Ruggieri 2005). A low volume (10 mL of water) extraction method was used to analyze pesticides in laboratory exposures. Higher volume samples were analyzed at SBU (900 mL of unfiltered water) and the USGS (247 mL of filtered water). Liquid-liquid extraction (water:hexane) used with volume ratios between 10:1 and 40:1 as described by Zimmerman et al. (2001) was a common feature of all three methods. It is advantageous to use hexane as an extracting solvent because it effectively partitions the targeted analytes but is inefficient at extracting potential interferents (as compared with methylene chloride or solid phase extraction (SPE) sorbents), and it can be evaporated without significant covolatilization of target

chemicals. The high performance liquid chromatography coupled to time-of-flight mass spectroscopy (LC-TOF-MS) and gas chromatography (GC) -MS based methods used were each able to sensitively and concurrently analyze all of the target analytes with very similar sensitivities (method detection limits of 1–3 pg injected for LC-TOF-MS, and approximately 10 pg injected for GC-MS).

Gas chromatography-MS analysis of pesticides (Zimmerman et al. 2001) was conducted either on a Hewlett Packard 5890 series II Plus GC equipped with a Hewlett Packard 5970 MSD (USGS) or on a HP 5890 series II GC equipped with VG Quattro mass spectrometer (SBU). The mass spectrometers were operated with electron impact ionization in selected ion monitoring mode (M/Z = 173, 73, 176, 123 and 123 as quantitation ions for malathion, methoprene, piperonyl butoxide (PBO), sumithrin and resmethrin, respectively). Terbuthylazine was used as a surrogate standard and *d*-10-phenanthrene as an internal standard. LC-TOF-MS analyses were conducted with a Micromass LCT, equipped with a Waters 2695 HPLC and a Z-spray electrospray ionization source. The mobile phase was methanol:water with gradient elution varying from 40% to 95% methanol in 12 min and held at 95% for 4 min. The aqueous mobile phase contained 10 μM sodium acetate, and all the pesticide analytes were analyzed as sodium adducts of parent molecules $(M + Na)^+$. Leucine enkephalin was added through postcolumn infusion to serve as an internal mass calibrant, to confirm analyte identification using accurate mass estimation (Benotti et al. 2003). The internal standards used included terbuthylazine and *d*-6-malathion. For GC-MS analysis, the final solvent volume was adjusted to 100 μL from which 2 μL was injected. For LC-TOF-MS analysis, the hexane extract was brought just to dryness with a gentle stream of nitrogen and the pesticides were redissolved in 100 μL of initial HPLC mobile phase (40:60 methanol:water), of which 10 μL aliquots were injected.

RESULTS

Acute Toxicity of Resmethrin, Malathion and Methoprene to Larval H. americanus

Preliminary experiments with resmethrin indicated that regardless of dose, little dose-dependent mortality was observed in less than 24 h (data not shown). Therefore, further experiments were performed after continuous exposures of 48–96 h. Survivorship for larval *H. americanus* exposed to resmethrin is shown in Figure 1. For larvae exposed to resmethrin for 48 h at 16°C, the LC50 was 0.26 μg L^{-1} (95% CI 0.18–0.38). After 96 h of exposure at the same temperature, control survival was lower, dropping from 88% at 48 h to 69%, as was the calculated LC50 at 0.095 μg L^{-1} (95% CI 0.075–0.114). When the 96-h exposure was repeated at 24°C, control survival was 76% and the LC50 was calculated at 0.10 μg L^{-1} (95% CI 0.09–0.12).

Resmethrin was significantly more toxic than malathion or methoprene to larvae. The 48-h LC50 for malathion at 16°C was calculated to be 3.7 μg L^{-1} (95% CI 3.3–4.2, Fig. 2), whereas methoprene exposure of up to 10 μg L^{-1} resulted in no significant mortality (Fig. 3).

Phenoloxidase Activity in Juvenile H. Americanus Exposed to Resmethrin and Malathion

It was difficult to obtain adequate hemolymph volumes from juvenile lobsters of this size. In general, only 100–200 μL of hemolymph could be obtained from all but the largest individuals.

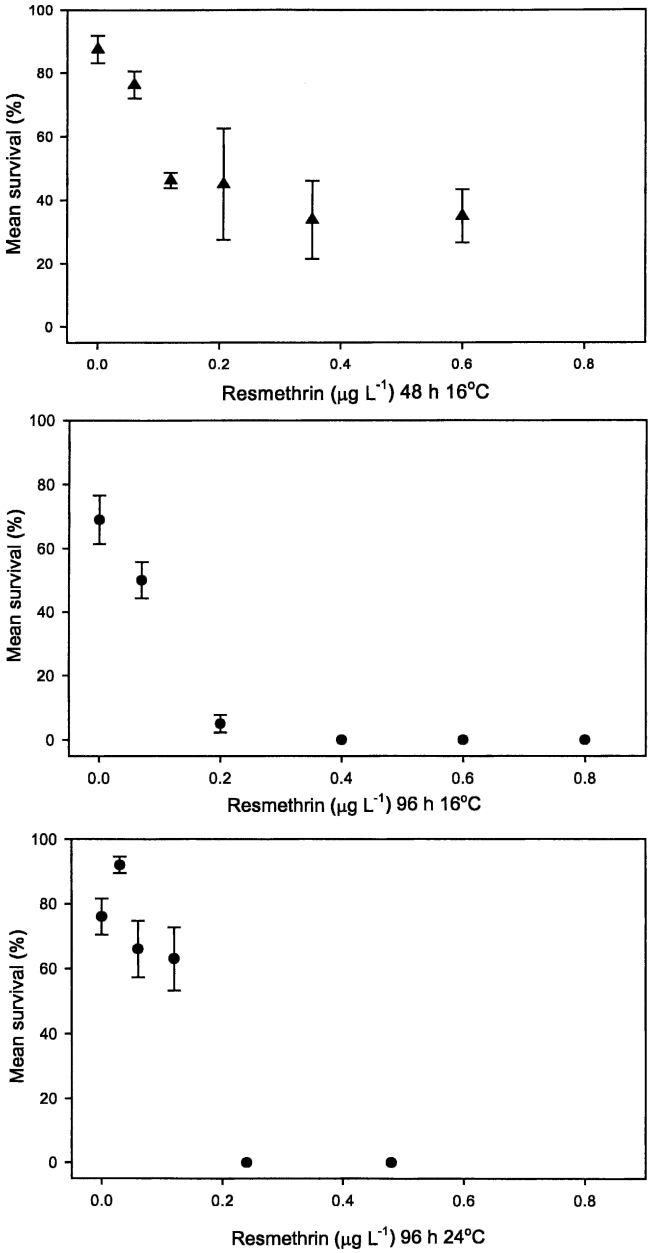

Figure 1. Acute toxicity of resmethrin to larval *H. americanus* after 48- or 96-hour flow through exposures, at non-stressful (16°C) or stressful (24°C) temperatures (mean ± SE); n = 4 replicates per treatment for 48-h exposure and n = 5 for 96-h; 20 larvae per replicate.

Although PO activity was not statistically related to size in the animals studied, activity was not detectable in samples from several of the smaller organisms. No significant relationship was observed between molt stage and PO activity (data not shown).

In experiments where animals were exposed to either 0.03 or 0.10 μg L^{-1} resmethrin at 16°C, PO activity was significantly reduced relative to controls at 0.03 μg L^{-1} but not 0.10 μg L^{-1} (Fig. 4). A somewhat surprising observation made during these experiments was that after 6 d of exposure, 2 of the 8 lobsters in the highest treatment group showed signs of locomotor impairment having trouble maintaining an upright posture with one flipped on its back. This inability to maintain normal posture was prevalent in

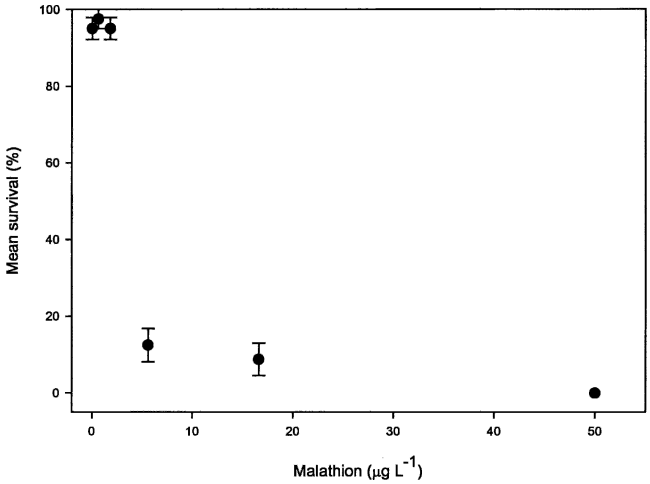

Figure 2. Acute toxicity of malathion to larval *H. americanus* after 48-h flow-through exposure at 16°C (mean ± SE); n = 4, 20 larvae per replicate.

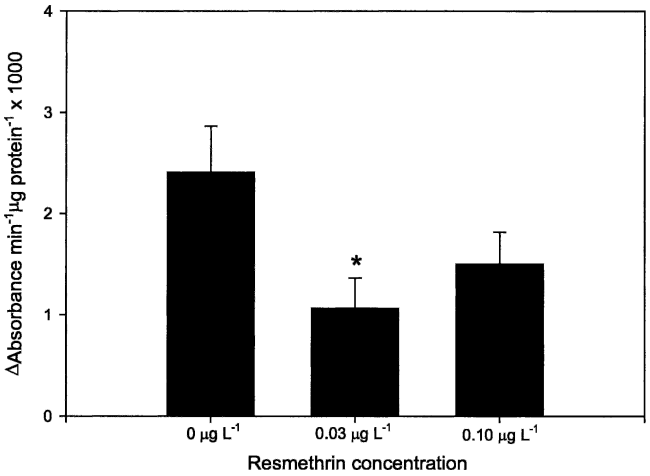

Figure 4. PO activity as a function of resmethrin exposure in juvenile *H. americanus* (mean ± SE, * P < 0.05 as compared to control response).

organisms exposed to higher resmethrin concentrations in preliminary experiments at higher doses ranging from 0.10–0.50 μg L^{-1}. Despite this, it should also be noted that no mortality of juvenile lobsters was observed at doses of 0.10 μg L^{-1} indicating that juvenile lobsters are less sensitive than larval lobsters to acute resmethrin exposure. In two additional experiments, done as part of an effort to assess the effects of temperature, no differences were observed between juveniles exposed to 0.03 μg L^{-1} resmethrin and control organisms (Fig. 5). Exposure to 1.0 μg L^{-1} malathion also failed to elicit a significant PO response. Temperature, however, did affect PO activity where a 65% increase was observed at the elevated temperature ($P < 0.006$).

Environmental Sampling

A combination of fresh water ponds, salt marshes, tidal inlets and embayments' and marine coastal waters (off Staten Island, NY) were sampled during 2002 (Table 1). The water depth at all of these sites was shallow (depth = 1–5 m). Estuarine or salt marsh samples were obtained after three spray events in 2003 (Table 2). The geographic location of all samples is shown in Figure 6. Malathion was not sprayed in the area during the years sampled and was not detected in any sample (detection limits of 0.1–0.5 ng L^{-1} by SBU and 5 ng L^{-1} by USGS). Note that field measurements are reported in ng L^{-1}, whereas toxicity results are referred to in μg L^{-1} (Tables 1 and 2).

In 2002, measurements made on Long Island surface waters at SBU detected resmethrin, the active ingredient in Scourge, at 5 out of 10 locations sampled within an hour after spray events. Concentrations detected ranged from 1.7–980 ng L^{-1}, but only one sample exceeded 150 ng L^{-1}. The pyrethroid sumithrin, found in Anvil, was not detected after two spray events. Methoprene, the active ingredient in the larvicide Altosid was detected after each of two spraying events at concentrations ranging from 7.4–631 ng L^{-1}. In contrast, the synergist PBO, included in the pyrethroid formulations Anvil and Scourge, was detected by SBU in all but two samples collected after spray events at concentrations ranging from 0.3–15,000 ng L^{-1}. Piperonyl butoxide was still present at three locations in samples taken 3 d after a Scourge spray after a period of heavy rain (Table 1, Map numbers 11–13). Resmethrin,

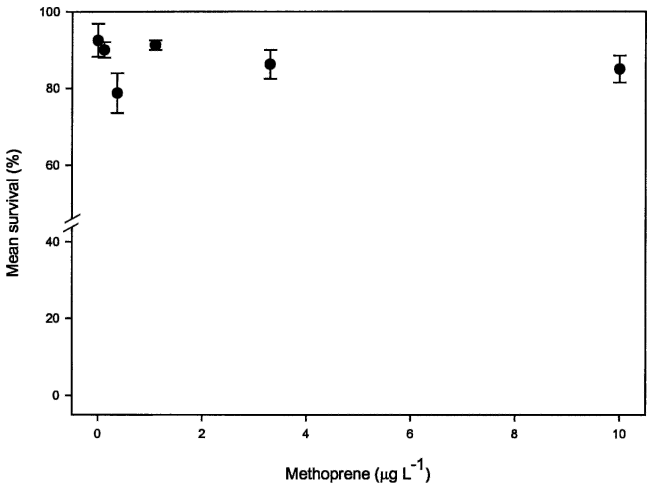

Figure 3. Acute toxicity of methoprene to larval *H. americanus* after 48-h flow-through exposure at 16°C (mean ± SE); n = 4, 20 larvae per replicate.

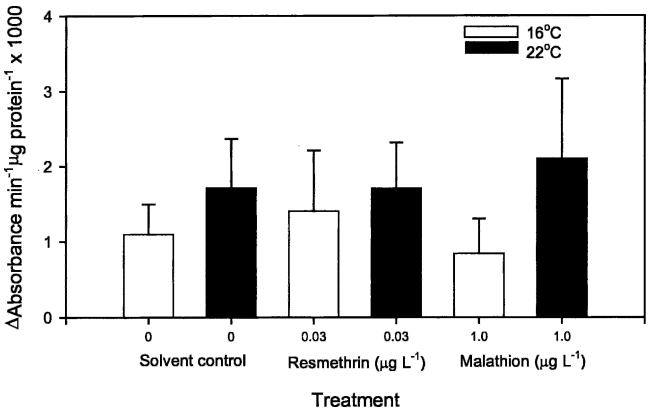

Figure 5. A comparison of the effects of temperature and pesticide exposure on phenoloxidase activity (mean ± 95% CI). The 16°C experiment was conducted separately from the 22°C experiment. The effect of temperature was significant (P < 0.006), however, the effect of the pesticide was not (P = 0.880).

TABLE 1.

2002 Sampling of surface water for pesticides.

						Concentration ng L^{-1}							
						Resmethrin		Sumithrin		Piperonyl Butoxide		Methoprene	
Spray Date	Sample Date	Product	Map Number	Location	Coordinates	USB	USGS	USB	USGS	USB	USGS	USB	USGS
6/18	6/18	Altosid	1	Carmen's River	40 46.300 N 72 53.617 W	—	ND	—	ND	—	ND	—	631
7/24	7/24	Altosid	2	Hecksher State Park	40 42.250 N 73 10.233 W	ND	ND#	ND	ND#	ND	ND#	7.39	ND
7/17	7/17	Scourge	3	Shinnecock Bay 1	40 46.300 N 72 43.600 W	ND	ND#	ND	ND#	ND	ND#	ND	ND#
				Shinnecock Bay 2		ND	ND#	ND	ND#	ND	ND#	ND	ND#
7/31	7/31	Scourge	4	Mastic 1	40 44.750 N 72 50.850 W	ND	ND	ND	ND	1.4	ND	ND	ND
				Mastic 2		ND	ND	ND	ND	0.59	ND	ND	ND
8/16	8/16	Scourge	5	Lake Ronkonkoma 1	40 49.950 N 73 07.567 W	—	ND	—	ND	—	ND	—	ND
				Lake Ronkonkoma 2		—	ND	—	ND	—	ND	—	ND
8/19	8/19	Scourge	6	Gibbs Pond	40 50.683 N 73 08.383 W	146	76	ND	ND	15,000	6,910	ND	ND
8/19	8/19	Scourge	7	Spectacle Pond	40 50.150 N 73 08.056 W	1.7	21	ND	ND	11	343	ND	ND
8/26	8/26	Scourge	8	Carl's River	40 42.100 N 73 19.717 W	5.49	18	ND	ND	120	41	ND	ND
8/26	8/26	Scourge	9	Trues Creek	40 41.867 N 73 16.933 W	980	293	ND	ND	3,200	13,400	ND	ND
8/26	8/26	Scourge	10	Sampawams Creek	40 41.800 N 73 19.067 W	ND	ND	ND	ND	25	35	ND	ND
8/26	8/29	Scourge	11	Sampawams SS after rain	40 41.949 N 73 18.926 W	ND	—	ND	—	290	—	ND	—
8/26	8/29	Scourge	12	Pine Lake SS after rain	40 41.461 N 73 16.652 W	ND	—	ND	—	210	—	ND	—
8/26	8/29	Scourge	13	Argyle Park SS after rain	40 41.741 N 73 19.889 W	ND	—	ND	—	230	—	ND	—
9/10	9/10	Scourge	14	Mastic Beach 1	40 44.850 N 72 50.967 W	6.2	ND	ND	ND	68	ND	ND	ND
				Mastic Beach 2		ND	—	ND	—	46	—	ND	—
9/10	9/10	Scourge	15	Pattersquash Creek	40 45.817 N 72 51.100 W	—	ND	—	ND	—	ND	—	ND
8/28	8/28	Anvil	16	Oakwood Beach SS	40 33.090 N 74 06.683 W	ND	—	ND	—	0.70	—	ND	—
8/28	8/29			Oakwood Beach SS after rain		ND	—	ND	—	0.64	—	ND	—
8/28	8/28	Anvil	17	Wolf's Pond SS	40 30.726 N 74 11.709 W	ND	—	ND	—	0.33	—	ND	—
8/28	8/29			Wolf's Pond SS after rain		ND	—	ND	—	0.31	—	ND	—

—: Not measured, ND: Not detected (USB detection limit 0.1 to 0.5 ng L^{-1}, USGS detection limit <5 ng L^{-1}, except ND# where ND <200 ng L^{-1}), SS: subsurface sample.

TABLE 2.

2003 Sampling of coastal water after Anvil spray events.

				Concentration ng L^{-1}	
Spray Date	Collection Date	Location	Coordinates	Sumithrin	Piperonyl Butoxide
		Hutchinson River			
8/13	8/14	Near storm drain 1	40 51.199 N 73 49.032 W	ND	1.0
		Near storm drain 2		ND	1.0
		Near pipe	40 51.091 N 73 49.037 W	ND	57
		In pipe		ND	15
		Long Island Sound Transect			
8/25	9/3	Station D-3 surface	40 59.318 N 73 24.318 W	ND	ND
		bottom		ND	ND
	9/3	Station T-1 surface	41 00.926 W 73 28.195 W	ND	ND
		bottom		ND	ND
	9/3	Station T-2 surface	40 57.601 W 73 33.572 W	ND	ND
		bottom		ND	ND
	9/4	Station T-3 surface	40 56.742 73 39.370 W	ND	ND
		bottom		ND	ND
	9/4	Station E-7	40 48.330 W 73 49.210 N	ND	0.22
	9/4	Riker's Island	40 47.759 W 73 52.475 N	ND	1.3
	9/4	Sunken Meadow	40 47.759 W 73 54.773 N	ND	6.6
		Time Series at Hammonasset State Park		mean (SD) n = 3	
9/22	9/22	Immediately after spray		1.1 (1.0)	20 (9.3)
	9/23	1 day after		ND	5.0 (4.1)
	9/24	2 days after		ND	2.5 (2.6)
	9/25	3 days after		ND	0.35 (0.03)
	10/2	10 days after		ND	0.29 (0.06)

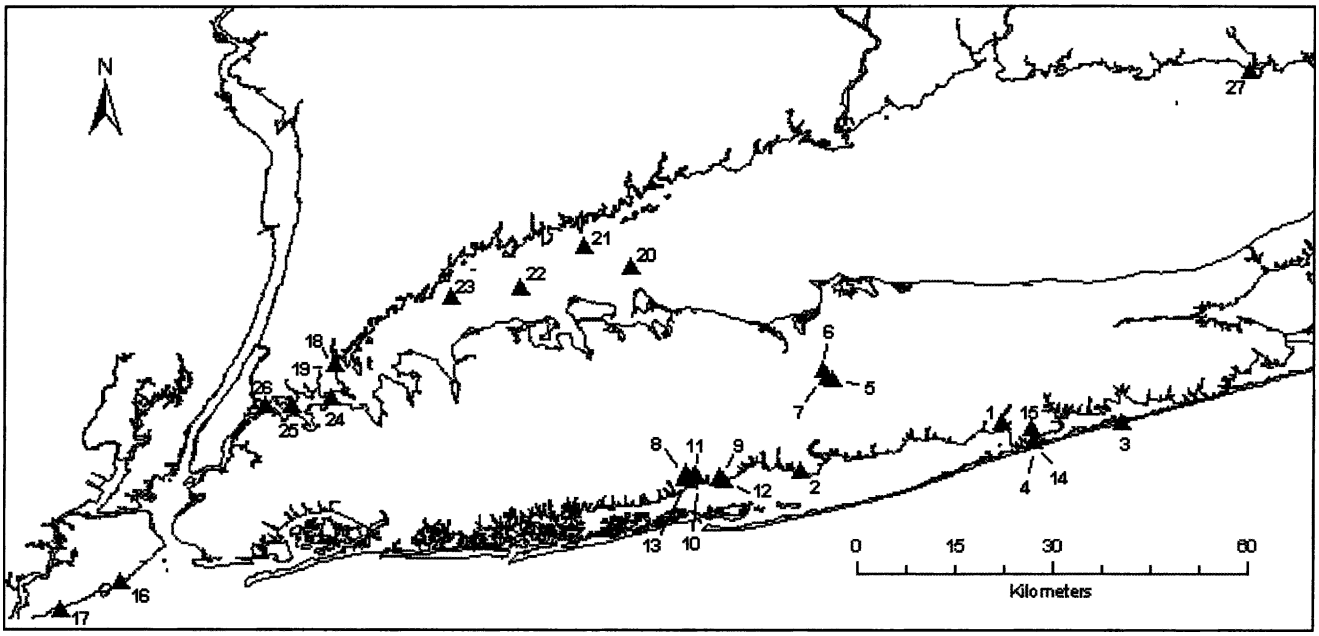

Figure 6. Field sampling locations in 2002 and 2003. See Tables 1 and 2 for coordinates and descriptions.

sumithrin, PBO and methoprene were simultaneously analyzed by the methods used. In no cases were pesticides detected other than those applied in an actual spray event, indicating that none of these compounds are persistent in the environment.

Generally good agreement was seen between samples collected at the same time and location and then analyzed at either the USGS or SBU. Pesticide levels reported by the two laboratories using different methods were generally within a factor of three of each other. At locations where more than one sample was collected and analyzed by the same method, very good agreement was reached, with values being within a factor of two of each other. There were no reported detections of pesticides by the USGS laboratory that did not correspond to positive detections by SBU using more sensitive methods.

Measurements made in the East River and the Narrows of far western LIS in September 2003 10 d after spraying failed to detect sumithrin, although PBO was found at concentrations ranging from 0.2–7 ng L^{-1} (Table 2). The highest concentrations of PBO were found in the samples collected from the stations farthest west and closest to the areas of known spraying. No methoprene (actively used in storm drains surrounding western LIS in 2003) was measured in LIS. Furthermore, no pesticides were detected in open surface waters of western LIS. After a truck spray of Anvil at Hammonasset State Park (Table 2), sumithrin was detected in a shallow creek in the salt marsh immediately after the spray event at a concentration of 1.1 ng L^{-1}, whereas PBO was found at concentrations 20 times higher. The next day, sumithrin was no longer detectable, but PBO at concentrations in excess of 2 ng L^{-1} was measured 2 d later, with detectable concentrations still present 10 d after spraying. Similarly, no sumithrin was detected in samples taken along the shore of the Hutchinson River (a tidal embayment in far western LIS) the day after aerial spraying of nearby Pelham Bay Park. PBO was detected in the same samples and was much higher in samples taken near tiled pipes that appeared to be storm drains emanating from the park.

DISCUSSION

American lobster larvae seem to be extremely sensitive to resmethrin with LC50s ranging from 0.10 $\mu g\ L^{-1}$ after 96-h exposures to 0.26 $\mu g\ L^{-1}$ after 48 h. Few other studies have investigated resmethrin's effect on aquatic invertebrates; however, Rand (2002) summarized a laboratory study that also conducted flow-through exposures with resmethrin and reported a similar 48-h LC50 of 0.22 $\mu g\ L^{-1}$ for the freshwater crustacean *Daphnia magna*. Burridge et al. (2000) conducted acute toxicity tests with larval and postlarval lobsters with another pyrethroid, cypermethrin, and found 48-h LC50s between 0.06 and 0.18 $\mu g\ L^{-1}$ using static-renewal exposures. Because LC50s tend to be reduced when using flow-through systems, we would expect the toxicity of cypermethrin to be greater than that reported here for resmethrin.

Raising the exposure temperature from 16°C to 24°C failed to significantly alter the LC50 for resmethrin; however, increased toxicity caused by the elevated temperature alone may have masked any additional pesticide effects. Lobsters have a wide temperature tolerance (−1–30.5°C) but generally inhabit areas that range from 5°C to 20°C (Lawton & Lavalli 1995). Several authors have shown that stage II lobster larvae were more sensitive to elevated temperatures than any other life stage (Gruffydd et al. 1975, Sastry & Vargo 1977). There has been little work, however, on the effect of temperature on pesticide toxicity. In their review of pyrethroid toxicity to aquatic organisms, Coats et al. (1989) reported several studies showing an inverse relationship between temperature and pyrethroid toxicity in both insects and trout. He attributed this to a combination of potential temperature-dependent interactions of pyrethroids on nervous tissue, or the influence of toxicokinetic parameters enhancing concentrations at the site of action at colder temperatures.

In this study, larval lobsters were more than 20 times less sensitive to malathion than to resmethrin with malathion's 48-h LC50 calculated at 3.7 $\mu g\ L^{-1}$. In contrast, De Guise et al. (2004) reported malathion 96-h LC50s for adult *H. americanus* derived

from single-dose static tests to be 10 times higher (38 μg L^{-1}). The acute toxicity of malathion and a number of pyrethroid pesticides to estuarine crustacea was reviewed by Cripe (1994). Results from flow-through tests with juvenile *Mysidopsis bahia* were similar to those reported here (2.6 μg L^{-1}), whereas 96-h LC50s derived from static tests with this and other estuarine crustacean species were generally higher ranging from 5–83 μg L^{-1}. The toxicity of various pyrethroids (cypermethrin, fenvalerate and permethrin) reported for similar species was generally 10–100 times greater than that reported for malathion, agreeing well with data reported here for resmethrin. In a field study, Conte and Parker (1975) reported that the water concentrations of 2–3.2 μg L^{-1} of malathion in surface water immediately after spraying most likely resulted in mass mortality of two shrimp species in marshes near Galveston Bay.

The juvenile hormone mimic methoprene found in Altosid was not found to be toxic to larval lobsters at concentrations as high as 10 μg L^{-1} after 48 h of exposure. These results are not surprising given methoprene's mode of action as a molting inhibitor. In the short-term studies measuring acute lethality, we had not really expected to see much toxicity with methoprene. It should be noted that the chemical formulation tested was a racemic mixture of *S* and *R* isomers, and previous work has shown the *S* isomers to be significantly more toxic than the *R* isomers (Celestial & McKenney Jr. 1994). However, even if the *R* isomers contributed little to observed toxicity, the racemic mixture would be expected to be approximately half as toxic as *S*-methoprene. In contrast to the results reported here, Walker et al. (2005) estimated static 72-h LC50s for *S*-methoprene for stage II lobsters to be only 2 μg L^{-1}. Results of other studies with crustaceans are in agreement with data reported here, indicating that methoprene has comparatively lower acute toxicity to aquatic crustaceans. Celestial & McKenney Jr. (1994) found elevated toxicity in mud crabs (*Rhithropanopeus harrisii*) exposed for up to 4 wk to *S*-methoprene at concentrations $\geq$100 μg L^{-1} in static renewal tests. Studies by this group found similar results under flow through conditions with mysid shrimp (McKenney Jr. & Celestial 1996). Finally, Wirth et al. (2001) reported no acute toxicity after 96 h to shrimp (*Palaemonetes pugio*) exposed to methoprene concentrations (no information was given on the isomer distribution) as high as 1,000 μg L^{-1}.

Mortality of larval lobsters in the control treatments ranged from 5% to 31%. Other investigators have reported similar outcomes in working with this species. Sprague and McLeese (1968) reported control mortalities nearing or exceeding 50% in 1- to 3-wk exposures. Wells and Sprague (1976) reported 21% overall control mortality in 96-h exposures and 51% in 30-d exposures. Burridge and Haya (1997) and Fiore and Tlusty (2005) both identified cannibalism as a source of control mortality when working with larval lobsters. It is possible, however, that this behavior differentially impacts the experimental treatments. Burridge and Haya (1997) noted that the larvae in controls and lower concentration treatments in exposures to pyrethroids remained active throughout the assay, whereas larval activity decreased at the highest concentrations tested. They also observed partially eaten larvae carcasses in some control treatments, but not in treatments with higher pyrethroid concentrations. Given that LC50s are calculated as a function of control mortality, this would serve to artificially increase the LC50 and underestimate a compound's toxicity.

Analysis of PO activity in pesticide-exposed juveniles indicated that neither resmethrin nor malathion significantly influenced the activity of this enzyme at sublethal levels. De Guise et al. (2004) reported that exposure to single or repeated doses of as low as 5 μg L^{-1} of malathion resulted in transitory reduction in phagocytic ability in adult lobster hemocytes. These effects were observed at doses of only 15% of the LC50 values determined under similar conditions. Although the exposure dose of malathion used in these experiments, 1 μg L^{-1}, was less than used by De Guise et al. (2004), it was a greater proportion of the LC50 determined in continuous exposures with larvae. It should be noted that De Guise et al. (2004) only reported sustained reduction in phagocytosis after 3 wk of repeated exposures to this dose. A more prolonged period of exposure may be necessary to detect a functional immune response in this species. Alternatively, PO activity in juvenile organisms may be too low to be responsive. Several of the smaller organisms used in this study had undetectable levels of PO activity. It would be interesting to compare levels observed in juveniles to that found in older/larger juveniles or adult organisms.

Several studies have reported decreased PO activity in crustaceans exposed to other environmental toxicants. Exposure of the common grass shrimp, *Crangon crangon*, to 50 μg L^{-1} of PCB15 reduced PO activity by a third as compared with control values, however, this study also reported mortality at this concentration that seemed to be approaching the 96-h LC50 (Smith & Johnston 1992). Another study by this group reported a reduction in PO activity in *C. crangon* exposed to harbor dredge spoils compared with reference animals exposed to clean sand, although the sample size was too small to support meaningful statistical analyses (Smith et al. 1995). Studies on the freshwater prawn, *Macrobrachium rosenbergii*, found a significant decrease in PO activity in as little as 48 h of sublethal exposure to 300 μg L^{-1} benzalkonium chloride (Cheng et al. 2003) and 100 μg L^{-1} copper sulfate (Cheng & Wang 2001). Both of these studies noted that PO activity continued to decrease with increasing exposure time to each compound at all concentrations tested, suggesting decreased PO activity as a possible explanation for an observed increase in susceptibility of the prawn to a bacterial pathogen (Cheng & Wang 2001, Cheng et al. 2003).

The combined effect of elevated temperature and exposure to either resmethrin or malathion also failed to produce a detectable change in PO activity, although elevated temperature itself caused a small but statistically significant increase in PO activity. Many other studies indicate that temperature can strongly influence immune response. Paterson and Stewart (1974) reported phagocytosis of sheep red blood cells by lobster hemocytes to occur more quickly at 10°C to 15°C than at 4°C. Steenbergen et al. (1978) found phagocytosis in lobster was reduced at temperatures above 22°C as compared with 20°C. Recently Dove et al. (2005) reported a large reduction in phagocytic activity and increased hemocyte count in adult lobster slowly acclimated to elevated temperatures (23°C as compared with 16°C). Chisholm and Smith (1994) surveyed seasonal variation in antibacterial activity and total hemocyte count in the shore crab *Carcinus maenas*, and found significant (>5-fold) depression in the ability of hemocytes to kill a marine bacterium at the low and high end of the temperature range for that year. Total hemocyte counts did not show this pattern. A more recent study by Hauton et al. (1997) did not show any seasonal trends in either parameter in the *C. maenas*, but did show a correlation between bacterial abundance in the water column and PO activity.

A more complete evaluation of the combined effects of temperature and pesticide exposure would benefit from additional measures of immune response. It is possible that some other mea-

sure of immune response, such as phagocytosis, may prove to be a more sensitive measure of immune suppression in young animals.

Field Measurements of Pesticides

Pesticides were detected at approximately 50% of the locations sampled directly after spraying. Detection of the pesticides' active ingredients was restricted to samples that were under aerial spray events and thus targeted for pesticide exposure. It was much more common to detect the synergist PBO even in surface waters that were removed (by spraying buffers, e.g.,) from areas of targeted spraying. The ratio of PBO to pyrethroid in surface water samples was always higher than that in the formulations of Scourge (resmethrin:PBO = 3:1) or Anvil (sumithrin:PBO = 1:1). These results indicate that there is either differential transport of active ingredient and synergist to surface waters or differential preservation in surface waters. Differences in transport to surface waters may occur either in the air after spraying or, in some cases, by tidal flooding or run-off from precipitation. Differences in preservation may occur as a result of the propensity of these two pyrethroids to be lost preferentially compared with the more water soluble PBO. It is believed that pyrethroids are lost rapidly from surface waters because of various processes including photochemical transformation and sorption to particles and sediments (Clark et al. 1989, Rand 2002).

In comparing the results of samples measured either as replicates or by both laboratories, it is important to recognize that results for replicate samples are from sequential samples, not duplicates splits from a larger sample. Recent studies (Brownawell & Ruggieri 2005) have demonstrated that pesticide concentrations from samples collected just after spraying at the surface and containing the air-water interface are significantly higher than concentrations from samples collected from just below the interface. Therefore, it is likely that pesticide distributions at the interface are somewhat patchy and poorly mixed, and this likely contributes to some variability in results between replicate samples.

The concentrations of methoprene, resmethrin and sumithrin measured in surface waters were usually lower, and often much lower than the levels found in this study to be acutely toxic to larval and juvenile lobsters. Of the samples analyzed, only 2 of them (Gibbs Pond and Trues Creek; Table 1, Map numbers 6 & 9) had concentrations of resmethrin approaching or exceeding those that were found to be toxic in this study. A simple hazard quotient (HQ) calculated as the ratio of resmethrin concentrations observed in the field to the lowest observable effects levels determined in the laboratory would indicate concern (HQ>1) only at these two sites. Those two samples were collected in a manner that included the air-water interface so much lower levels would be expected to mix lower into the water column. Furthermore, detectable levels of measured pyrethroids were found only in enclosed or semienclosed bodies directly under the path of aerial pesticide application, and not in nearshore coastal waters where lobsters would be found.

The results from this study suggest that pesticide levels in surface waters after spraying are low, compared with toxicologically significant exposures, under a range of typical applications. However, it remains difficult to assess the possible levels of pesticides that might have been delivered to western LIS after tropical storm Floyd in 1999, caused by the large number of applications in the watershed preceding the storm, the unusually large amount of rain deposited that might have facilitated enhanced run-off, and the rapid mixing of LIS from the storm.

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