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An Overview of Mosquito Control Practices, the Risks, Benefits, and Nontarget Impacts, and Recommendations on Effective Practices that Control Mosquitoes, Reduce Pesticide Use, and Protect Wetlands.

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The Xerces Society for Invertebrate Conservation is a nonprofit organization that protects wildlife through the conservation of invertebrates and their habitat. Established in 1971, the Society is at the forefront of invertebrate protection, harnessing the knowledge of scientists and the enthusiasm of citizens to implement conservation programs worldwide. The Society uses advocacy, education, and applied research to promote invertebrate conservation.

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Front Cover Photograph

Saltmarsh at Cape May, NJ. Photograph by Celeste Mazzacano/The Xerces Society.

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EXECUTIVE SUMMARY

Humans and mosquitoes have a long and contentious history. For centuries, people have tried to protect themselves, their pets, and their livestock from the irritation of nuisance biting and the effects of the disease organisms some mosquito species can transmit. Early organized efforts to control mosquitoes were framed in terms of a war of annihilation and implemented via a scorched-earth policy of drained and oiled wetlands and toxic broad-spectrum pesticides, the effects of which still linger in our landscape today.

Many currently used mosquito control agents are narrower spectrum and less toxic than those used in the past, but their use still has significant negative impacts on many aquatic invertebrates as well as the fish, birds, and amphibians that live and feed in wetlands. Increased understanding of wetland ecology, biodiversity, and food webs—and of the life histories of the mosquitoes themselves—combined with development of pest management practices in which application of least-toxic pesticides is done only as a last resort have made more ecologically friendly integrated mosquito management possible.

This report reviews current mosquito control practices in the United States, describes risks and benefits associated with different types of mosquito control—including direct and indirect impacts of chemical and biological controls on nontarget organisms—and provides recommendations on how to develop effective practices to manage mosquito populations while reducing pesticide use and conserving wetlands.

Mosquito control is done using agents that kill the adult (adulticides) or immature (larvicides) form of the insect. The most commonly used adulticides are organophosphate (e.g., malathion, naled) and pyrethroid (e.g., pyrethrin, permethrin, resmethrin, sumithrin, prallethrin) insecticides. These compounds have broad-spectrum toxicity and cause severe impacts to nontarget invertebrates, fish, amphibians, and birds. They have been implicated in declines in both wetland-associated and terrestrial wildlife, including endangered species that live near treated areas.

Mosquito larvicides include compounds that disrupt larval development, such as methoprene and diflubenzuron; microbial agents such as *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* that are toxic to mosquito larvae when ingested; and surface oils such as Golden Bear that interfere with the larva's ability to breathe. These larvicides are recognized as being relatively nontoxic to nontarget organisms (although methoprene and diflubenzuron have documented direct impacts on nontarget invertebrates), but such direct toxicity studies rarely, if ever, address indirect effects. For example, formulations of Golden Bear oil have been shown to disrupt development and cause malformations of duck eggs. Biopesticides such as Bti are highly toxic to true flies (Diptera), which includes a variety of

Millions of people in the United States live close to wetlands. The management of these areas affects not only neighbors but also the myriad wildlife living in and around them. (Photograph: Celeste Mazzacano/The Xerces Society.)

organisms that are an important food source in wetlands such as non-biting midges, shore flies, and gnats. Widespread and repeated Bti applications thus have the potential to severely disrupt local food webs and change wetland community composition. Chemical and biological pesticides are also formulated with adjuvants and carrier agents that may have additional negative effects on aquatic systems and nontarget organisms; however, because these compounds are "inert ingredients," they are not examined in acute toxicity tests.

Many fish, birds, and amphibians rely on aquatic flies as an important food resource in the water, and the winged adult forms of aquatic insects can provide 25–100% of the energy or carbon resources for terrestrial consumers such as bats, lizards, and birds. Aquatic macroinvertebrates that develop in pesticide-laden waters can act as "biotransporters" of contaminants because their accumulated pesticide load is taken up by the predators that consume them. Declines in aquatic invertebrate populations due to pesticide impacts also have serious implications for the energy budget of the aquatic and surrounding terrestrial ecosystems. Reduction of the aquatic insect food base can also impact nest-site choices of female ducks as well as the food resources available to sustain ducklings and migrating waterfowl.

This report will help land managers by providing solutions to mosquito issues that are both more effective and less toxic to the aquatic ecosystem.

Impacts of Pesticides on Nontarget Animals

Modern mosquito control agents cause significant negative impacts to nontarget animals. These impacts may be due to direct toxicity, whether acute or via exposure to sublethal concentrations, or they may be indirect, occurring at the level of altered wetland community composition and food web effects by having an impact on food for fish and birds. Documented impacts include:

Organophosphates: temephos (Abate), malathion, and naled.

- Organophosphates have broad-spectrum toxicity and negatively impact many aquatic organisms, including fish, dragonfly and damselfly nymphs, mayfly nymphs, water boatmen, microcrustacea, and non-biting midges. Drift from ultra-low volume sprays used against adult mosquitoes affects pollinators and butterflies, and low-flying aircraft used in spraying can disturb nesting birds.

Pyrethroids: permethrin, resmethrin, d-phenothrin (sumithrin), and bifenthrin.

- Pyrethroids are highly toxic to many aquatic organisms, including mayflies, stoneflies, caddisflies, and crustaceans. Drift from ultra-low volume sprays used against adult mosquitoes affects pollinators and butterflies, and low-flying aircraft used to deliver sprays can disturb nesting birds.

Surface oils and films: monomolecular films (Arosurf, Agnique), mineral-based oils (BVA2), and petroleum oils (Golden Bear).

- Monomolecular oils and films create a barrier at the air/water interface that suffocates invertebrates that breathe atmospheric oxygen at the water's surface, including mayfly nymphs, microcrustacea, and aquatic bugs and beetles. Many of these are also important predators of mosquito larvae, which may reduce mosquito control by natural enemies. Oils can reduce hatching success of bird eggs and impair thermoregulation and foraging in ducklings.

Insect growth regulators: juvenile hormone mimics (methoprene [Altosid]) and chitin synthesis inhibitors (diflubenzuron [Dimilin]).

- Insect growth regulators are broadly toxic to insects and other invertebrates, especially crustaceans. Chronic effects of methoprene growth regulators include developmental disorders, morphological defects, and reproductive anomalies in dragonfly nymphs, mayflies, beetles, crustaceans, and non-biting midges. Methoprene may be linked to abnormalities that occur during in metamorphosis in amphibians.

Bacteria: *Bacillus thuringiensis* var. *israelensis* (Bti; Vectobac, Aquabac, Bactimos, Summit, Teknar), *Bacillus sphaericus* (Bs; Vectolex), and *Saccharopolyspora spinosa* (Spinosad; NATULAR).

- Bti is toxic to non-biting midges, which comprise a large proportion of the animal biomass in wetlands and are an important food resource for aquatic invertebrates, fish, amphibians, bats, waterfowl, wading birds, and some passerine birds.

Larvivorous fish: *Gambusia* (mosquitofish).

- Widespread introduction of *Gambusia* into habitats where they are nonnative has devastating effects on native fish and amphibians. Their generalist feeding habit can reduce abundance of natural enemies of mosquitoes in the habitat and lead to increased mosquito numbers.

Effective Mosquito Control that Reduces or Eliminates Pesticide Use

Public Education
Many wetlands produce few or no mosquitoes, but a variety of human-made habitats—such as stagnant backyard bird baths, clogged gutters, unmaintained ponds, and neglected pet dishes—serve as fertile breeding grounds for mosquitoes. Consequently, community-based mosquito management programs founded in public education and community involvement that focus on individual actions to remove such breeding sites have been found to be the best means to achieve effective mosquito and disease control. Follow-up surveys to determine overall success of public education, as well as focusing on specific groups or demographics that require a more tailored or alternative approach, are critical for success.

Interagency Cooperation
Federal, state, county, or city agencies can have conflicting wetland management goals, with wetland managers concerned with the effects of mosquito control practices on wildlife health and diversity and mosquito control agencies fearing increased production of mosquitoes from wetlands managed as natural sites. An interdisciplinary approach may require recognizing that the goal of a natural resource agency to maintain biodiversity is not in accord with the goal of a mosquito control agency to remove nuisance-biting mosquitoes, and both must work together to achieve mosquito management that provides necessary control when needed with the fewest negative impacts on the habitat.

GIS-Based Surveillance
Factors that strongly affect mosquito development such as water, vegetation, and surrounding land use can be identified from available remotely sensed data and used to develop locally or regionally targeted control plans for different mosquito species. GIS surveillance provides more comprehensive mapping than is possible on the ground, especially with limited staff and resources, and can enable identification and targeted treatment of "hotspots" where mosquito production is a true problem.

Site-Specific Knowledge
People often assume that all wetlands produce nuisance or disease-carrying mosquitoes, but healthy wetlands with a diverse community of aquatic invertebrates, fish, amphibians, and birds that prey on mosquito larvae often produce few to no mosquitoes. Regular monitoring is critical to determine whether mosquitoes are emerging from a site and if so, whether the species prefer to feed on humans, present a nuisance-biting issue as opposed to a public health risk, and if they have flight capability that enables them to disperse far from the site into residential areas. Detailed knowledge of site topography, hydrology, precipitation, and vegetation can identify microhabitats producing the greatest number of mosquitoes that can be targeted for physical or chemical spot-control when needed, reducing mosquito abundance while leaving a majority of the habitat untreated.

Conservation of Natural Enemies
Invertebrates that prey on mosquito larvae include dragonflies and damselflies, beetles, true bugs, predatory flatworms, and some aquatic crustacea such as tadpole shrimp and copepods. These predators occur naturally in wetlands, and many have life stages that can rapidly colonize newly-flooded sites. Spiders, bats, amphibians, fish, and birds can also consume mosquitoes. All of these animals are generalist predators; they do not target mosquitoes specifically, but studies show their presence in a wetland can reduce and even completely control mosquito populations. Mosquito management practices that conserve natural enemies can reduce mosquito numbers while protecting the food chain, sustaining an intact and diverse biotic community, and conserving rare or endemic species in the habitat.

Vegetation Management
Constructed wetlands such as those used in stormwater management can be designed and constructed with features that significantly reduce the ability of the site to produce mosquitoes. Constructed wetlands that are steep-sided, have less than 20% of the basin covered by vegetation, and provide for different levels of water and flow rates, including deeper pools where natural enemies can establish, are linked to decreased mosquito production and can create sites where additional mosquito control is rarely needed. Vegetation management done to improve habitat for waterfowl can also be tailored to sustain waterfowl while reducing mosquito numbers and increasing the abundance of other invertebrates eaten by waterfowl.

Bait Traps
Attractant-based traps for "attracticide" (lure and kill) mosquito control are still being investigated. Results vary greatly depending on location, habitat, and mosquito species, but bait traps have some potential to reduce mosquito abundance, especially in areas where one species dominates the population or where adult mosquitoes do not disperse far from the larval habitat.

Recommendations for Ecologically Sound Mosquito Management

An optimal approach to ecologically sound mosquito management requires consideration of several key interconnected elements. No single mosquito management plan will have equal efficacy at all sites, but the recommendations below will enable formulation of a mosquito management plan that is tailored to the individual needs and characteristics of a site while balancing the needs of the environment with those of the human community.

1. **Educate the public.** An informed public is critical for mosquito management. The surrounding community should know whether a wetland is producing mosquitoes at all, and if so, what their dispersal capacity and human biting preference is, the risk of contracting mosquito-borne disease, and personal protective measures that should be taken to prevent being bitten. Effective ongoing education regarding elimination of breeding sites in residential and urban areas where many of the human-associated, container-breeding mosquito species that can transmit disease pathogens occur has a large impact on public health. Explanation of site-specific mosquito management actions and their importance in protecting wetland health, biodiversity, and food webs while minimizing or eliminating pesticides will generate greater understanding of why "zero tolerance" for mosquitoes should not be practiced.

2. **Monitor consistently and thoroughly.** Monitoring is essential to determine whether a site is producing mosquitoes in significant numbers and to identify the species produced, assess seasonal patterns of abundance, and pinpoint microhabitats that are hotspots of mosquito production. If a plan is in place that involves pesticide use after defined mosquito abundance is reached, monitoring is essential to determine when that threshold has been exceeded, and to determine the efficacy of any treatments. Monitoring should also be done to assess the suite of natural enemies present at a site and their relationship with seasonal mosquito abundance, and to determine whether sensitive species that will be harmed by insecticide use are present.

3. **Form cooperative partnerships.** Ecologically sound mosquito management requires extensive knowledge of wetlands, their wildlife communities, mosquito species and life history, and public health, as well as ongoing education, monitoring, and surveillance. It is unlikely that any single entity will encompass all the necessary expertise, and working partnerships with several organizations will provide needed skills and resources. Because site-specific management practices may be implemented within a larger framework of existing local mosquito control, extensive communication and cooperation with regional vector control agencies is also required.

4. **Determine existing local mosquito control methods.** The existence and type of mosquito control efforts in the area and their compatibility with a desired site-specific management plan must be determined.

5. **Create informative maps.** Accurate maps that display and correlate multiple layers of GIS-based data are a powerful tool in mosquito management. Mapping the data from ongoing monitoring in conjunction with habitat characteristics, vegetation, topography, rainfall, and temperature facilitates identification of potential mosquito-producing hotspots that can be targeted for treatment, seasonal patterns in mosquito production, areas where natural enemies and sensitive species may be present, and portions of habitat that may be amenable to manipulation to reduce mosquito breeding (where appropriate).

6. **Implement a site-specific management plan.** An effective site-specific mosquito management plan is based on integrated management, ongoing monitoring, detailed knowledge of the life history of mosquito species produced at a site, and management practices that improve site quality and sustain increased biodiversity, including natural enemies of mosquitoes. Once a public education campaign is completed, decisions must be made as to whether the political and/or social climate will allow a desired outcome of "no treatment" for nuisance mosquitoes, threshold levels of abundance that will trigger treatment of vector species in a situation of documented public health risk, and the best combination of least-toxic alternatives to use when treatment is necessary.

7. **Implement Regulations Requiring Permits.** The EPA should fully implement new national regulations requiring permits under the National Pollutant Discharge Elimination System for application of chemical and biological pesticides in and around wetlands and other water bodies to control mosquitoes. Nationally, over 350,000 pesticide applicators, including the city, county, state, and federal governmental agencies that conduct most mosquito control programs, are now required to find ways to reduce pesticide use and adopt Integrated Pest Management practices.

1 Diversity and Ecological Importance of Mosquitoes

Mosquito Diversity

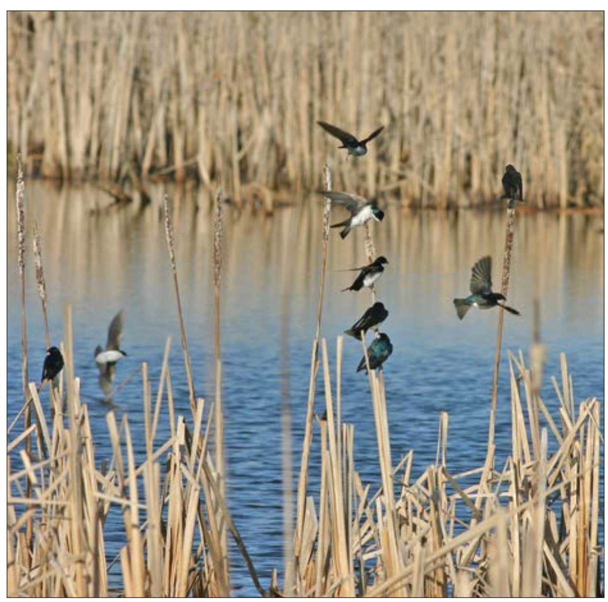
Just over 170 of the nearly 3,500 described species of mosquito in the world are found in the United States (Wallace & Walker 2008), including both native and introduced species. Many mosquito species do not feed on humans, some do but are nothing more than nuisance biters, and a few have the ability to act as vectors, i.e., to transmit disease pathogens that affect the health of wildlife, humans, livestock, or pets. Although no one enjoys being bitten by a hungry mosquito, only a small proportion of mosquito species are pests. Species best known for their impacts on human health are primarily in the genera *Aedes*, *Anopheles*, and *Culex*. Each species has its own particular life history, habitat preference, and dispersal ability (see Appendix A for more information), which means there will never be a "one size fits all" approach to mosquito control.

Importance of Mosquitoes in Wetland Ecology

Given the long history of negative interactions between humans and mosquitoes, it can be easy to forget that these insects play important roles in wetland ecology. The filter-feeding behavior of mosquito larvae on microorganisms, phytoplankton, and particles of organic detritus (reviewed in Merritt et al. 1992) plays a large role in nutrient cycling in wetland habitats, and mosquito larval abundance in temporary pools is thought to have a strong influence on ecosystem structure and processes such as primary productivity and nutrient cycling (Mokany 2007). Larval and adult mosquitoes are an abundant food source for a variety of aquatic insects and other invertebrates as well as for fish, amphibians, lizards, and birds, including migrating and breeding waterfowl. Mosquitoes are known pollinators of the bluntleaved orchid (*Habenaria* (*Platanthera*) *obtusata*) (reviewed in Kevan et al. 1993), but it is unclear whether their nectar-feeding habits also allow them to incidentally pollinate other plants.

Although a wide range of studies have focused on mosquito control, few have addressed the importance of mosquitoes in wetland biodiversity. A recent literature review (Dale & Knight 2008) found significant information gaps in the role of mosquitoes in wetland ecology, as well as the long-term impacts of larval mosquito control on nontarget organisms; the authors also noted that the potential of mosquitoes to make a positive contribution to overall wetland biodiversity is a novel and little-considered concept (but see Schäfer et al. 2004).

Because wetlands in a given region can vary greatly in hydroperiod and invertebrate community composition, it is important to recognize that some wetlands do not produce nuisance or vector mosquitoes at all. A study in Madison, WI, to assess disease risk from *Culex* mosquitoes found that only 25% of the 521 natural and constructed urban wetlands sampled across two years contained *Culex* larvae (Irwin et al. 2008), and pinpointed a small number of stormwater ponds as mosquito "superproducers." Very few mosquito larvae were present in most of the microhabitats surveyed in a large wetland complex in Iowa, and the main production of nuisance-biting and vector mosquito species occurred in microhabitats with intermittently flooded vegetation (Mercer et al. 2005). Some researchers stress the importance of considering mosquitoes within the larger conceptual frame of the many biotic and abiotic factors that influence their abundance, to better understand and predict annual population variations (Chase & Knight 2003; Knight et al. 2004; Juliano 2007).



Mosquitoes lie at the base of a food web that supports a wide diversity of larger and, to many people, more attractive animals. (Photograph: © Michel Bordeleau.)

2 Mosquito Control Past and Present

History of Mosquito Control in the United States

The negative impacts of mosquitoes on humans and livestock have been recorded for hundreds of years. Even before the ability of some mosquitoes to act as vectors (transfer disease pathogens) to humans was known, wetlands were considered places that bred disease (as well as an abundance of insect life). Writers as early as Hippocrates attributed the cause of malaria as drinking stagnant water (McNeill 1976), for example, and the journals of Lewis and Clark contain numerous uncomplimentary references to mosquitoes. Some have even hypothesized that the combined effects of chronic malaria and the medicines taken to combat the disease may have contributed to Meriwether Lewis' suicide three years after his expedition (Danisi & Jackson 2009).

Mosquitoes were seen as an inescapable source of misery in the United States for centuries, impacting agriculture, business, real estate, and recreation. In the early 1900s, the idea of community-level mosquito control led to the development of public health programs in Florida, California, and New Jersey. Organizations with names such as the New Jersey Mosquito Extermination Association and the Florida Anti-Mosquito Association (Patterson 2004) reflected a desire for total eradication of mosquitoes. Indeed, the organization that ultimately gave rise to the American Mosquito Control Association was first created in 1903 as the National Mosquito Extermination Society. Leland Howard, who headed the USDA Bureau of Entomology from 1894 to 1927 and did pioneering work in the control of mosquitoes and malaria in the United States, declared that "mosquito extermination is not a temporary interest but the beginning of a great and intelligent crusade." (Patterson 2009).

Early mosquito control measures were often drastic, with broad and dramatic consequences. Campaigns in this crusade included spreading crude oil, kerosene, or diesel oil across the surface of water bodies to suffocate mosquito larvae, and using broad-spectrum poisons such as Paris green, which is made with copper acetoarsenite, a deadly toxin (Dale & Hulsman 1990; Patterson 2004, 2009; Floore 2006). Pyrethrum oil, a natural pesticide isolated from chrysanthemums, was also used as a mosquitocide. Extensive ditching and draining was done in wetlands too large to be oiled, such as coastal salt marshes. Up to 95% of Atlantic coastal salt marshes were ditched in the first half of the twentieth century (Clarke et al. 1984; Crain et al. 2009), causing permanent habitat alterations whose effects are still being remediated in the landscape today. However, people began to realize that these chemical and physical controls caused substantial harm to wildlife and plants and were moreover reaction-

Photographs showing the Works Progress Administration's early efforts at mosquito control capture how methods have changed over the decades. In this photograph, taken in 1941, WPA workers in Louisiana are spraying fuel oil in a ditch. (Photograph: WPA archive, http://nutrias.org/photos/wpa/images/35/350210.jpg.)

ary, doing little to control the source of the problem. At the same time, agricultural irrigation development and dam construction created new habitats for mosquito breeding. Continuing investigation of mosquito species in the United States, including works such as *Mosquitoes of New Jersey* (Smith 1904), *Mosquitoes of Florida* (Byrd 1905), and *Mosquito Life* (Mitchell & Dupree 1907), along with scientific investigations into diseases such as malaria and yellow fever, led to the realization that larval habitat preferences, adult dispersal ability, and disease vector capacity differed among different species of mosquito, and that mosquito control could thus become a more targeted affair.

The mid-1900s saw the rise and fall of a variety of synthetic pesticides for mosquito control, many of which are no longer registered for use in the United States due to their severe environmental impact, as well as intensified research to find compounds that repel mosquitoes from biting (Floore 2006; Patterson 2009). Potent, persistent organochlorine (OC) pesticides such as DDT (dichloro-diphenyl-trichloroethane), lindane, chlorpyrifos, and dieldrin were used against larval and adult mosquitoes. The rapid development of resistance in mosquito populations caused these pesticides to be discontinued, although their residues persist in soil and wildlife. OCs were followed by organophosphate insecticides (OPs) such as malathion, temephos, fenthion, methyl parathion, and methoxychlor, but mosquitoes quickly exhibited resistance to these compounds as well (Rathburn & Boike 1967), and most were highly toxic to other wildlife. In the wake of mosquito resistance to OCs and OPs, development of synthetic pyrethroids, which are analogs of the naturally-occurring pyrethrin pesticides found in chrysanthemums, was undertaken. The 1900s also saw the use of natural enemies for mosquito control, as the ability of so-called mosquitofish, *Gambusia affinis*, to

Acceptance of the wide use of insecticides for mosquito control was so entrenched in public culture that even automotive adverts featured them.

consume large numbers of mosquitoes was noted. From 1905 through the 1920s, *Gambusia* were introduced for mosquito control in states such as Florida, Hawaii, New Jersey, Mississippi and California (Pyke 2008; Patterson 2009) and were an established tool in the vector control toolbox by the early 1920s, even though multiple investigators found that in many cases, indigenous fish species provided superior mosquito control (Pyke 2008).

Mosquito Control Today

A survey by the American Mosquito Control Association found that by 1997, there were at least 345 mosquito control districts or programs in the United States, conducting operations at an estimated annual cost of over $231 million, in areas affecting over 97 million people (ASTHO 2005). Many modern insecticides used to control mosquitoes are much less damaging to the environment than those previously used, but the use of any pesticide is accompanied by impacts to nontarget organisms and aquatic ecosystems. With the development of widespread resistance to chemical treatments among mosquito populations and agricultural crop pests and recognition of the environmental impacts of pesticide use, entomologists began to craft multi-pronged control plans for pest insects based in Integrated Pest Management (IPM). IPM is intended to substantially reduce reliance on chemical controls by using a suite of different targeted control methods. The basic tenets of IPM stress that eradication of a pest insect is not the goal. Rather, control measures are instituted only after regular surveillance determines that pest levels have risen to an economically damaging level (or, in the case of mosquitoes, a level at which public health is likely to be compromised), and the natural system is to be preserved undisturbed as far as possible. An IPM program is envisioned as a sustainable approach combining cultural, physical, biological, and least-toxic chemical control strategies. Similar plans used by mosquito control agencies may be referred to as Integrated Mosquito Management (IMM) or Integrated Vector Management (IVM). The American Mosquito Control Association defines IMM as "a comprehensive mosquito prevention/control strategy that utilizes all available mosquito control methods singly or in combination to exploit the known vulnerabilities of mosquitoes in order to reduce their numbers to tolerable levels while maintaining a quality environment" (AMCA 2009), and confirms that the concept and practice of IMM does not emphasize mosquito eradication. Xerces and other environmental organizations further stress that mosquitoes are a natural and important part of many aquatic ecosystems. Their eradication is neither necessary nor beneficial for the environment, and is moreover an unrealistic goal. (Note: for the sake of clarity, all future mention of IPM, IVM or IMM will be referred to as integrated management).

In theory, integrated management provides mosquito control using methods that are sensitive to local species and habitat conditions and targeted to known sources of mosquito production during appropriate developmental stages (reviewed in Lacey & Orr 1994). As no single method is effective against all species in all regions, detailed knowledge of local species and their life histories and habitat preferences is required. Another critical component is regular surveillance to monitor abundance, ascertain when a number corresponding to a stated action threshold for treatment has been attained, and assess the efficacy of treatment. An active integrated management program often incorporates pesticides, but these are intended to be used only when absolutely necessary as one of many ongoing methods of mosquito control, using the least-toxic alternative applied in such a way as to cause the minimum harm to nontarget organisms. Regular programmatic scheduled insecticide treatment is contrary to the tenets of integrated management, regardless of whether chemical or biological agents are used. Some workers argue that to be truly successful, integrated management decisions must be made by partners at the local level, because mosquito populations and mosquito-borne disease risk will both vary with, and be affected by, local community conditions (van den Berg & Takken 2007). A World Health Organization position statement on integrated management stresses that different practices have the potential to be either synergistic or antagonistic, and urges careful consideration of combinations of integrated management practices, along with ongoing evaluation of efficacy, appropriateness, and sustainability of management plans (WHO 2008). In practice, a true integrated management program may require financial resources and/or entomological expertise beyond the capacity of many mosquito control agencies. Consequently, ongoing pesticide application at regular time intervals during peak seasons for mosquito production—which is counter to integrated management practices—is done as a fallback.

A position paper from the Society of Wetland Scientists (SWS 2009) provides a review of current practices in mosquito control, but begins with a disclaimer that SWS does not endorse the use of any of the specific management techniques described, and concedes "…we recognize that wetland management for mosquito control may be at odds with management for other important goals such as maintaining biodiversity." Most mosquito control agencies in the United States use a relatively standard palette of pesticides to control the aquatic larvae or winged adult stages of the insect. Pesticides directed against larval mosquitoes (larvicides) are applied to the aquatic habitat and are available in a variety of formulations, including pellets, granules, briquettes, and liquids. Pesticides used to control adults (adulticides) are generally applied as an ultra-low volume (ULV) spray in terrestrial areas, creating fine aerosol droplets that kill flying mosquitoes on contact. Adulticiding is widely recognized as being less effective than larval control because adult mosquitoes may disperse long distances from the sites where they developed and can continue to emerge for several days from a local mosquito-producing site. Adulticiding is, thus, a short-term solution that does not treat the source of the problem.

Pesticides commonly used against mosquitoes are included in the list of control methods below (and are described in detail in chapter 4).

- Organophosphates: malathion, naled, temephos (Abate).
- Pyrethroids (synthetic derivatives of naturally-occurring pyrethrins): bifenthrin, d-phenothrim (Sumithrin), permethrin, resmethrin; these are frequently mixed with the synergist piperonyl butoxide (PBO), which interferes with a mosquito's ability to detoxify the pyrethroid.
- Surface oils and films:
  - monomolecular films such as Agnique, Arosurf;
  - mineral-based oils such as BVA2;
  - petroleum oils such as Golden Bear
- Insect growth regulators:
  - juvenile hormone mimics such as methoprene (Altosid);
  - chitin synthesis inhibitors such as diflubenzuron (Dimilin).
- Biological controls:
  - bacteria such as *Bacillus thuringiensis* var. *israelensis* (Vectobac, Aquabac, Bactimos, Summit, Teknar), *Bacillus sphaericus* (Vectolex), and *Saccharopolyspora spinosa* (Spinosad; NATULAR);
  - fungi such as *Lagenidium giganteum*;
  - larvivorous fish such as *Gambusia*.

3 Legislation Affecting Mosquito Control

National Legislation

Federal Insecticide, Fungicide, and Rodenticide Act

The earliest pesticide laws established in the United States were mainly designed to protect farmers from being sold substandard products (Federal Insecticide Act of 1910) and to protect consumers from residues on foods (The Pure Food Law, as amended in 1938) (Ware 2004). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was established in 1947; it superseded the 1910 Federal Insecticide Act and was used primarily to regulate pesticide labeling (FIFRA, 7 U.S.C. §136 et seq., 1947). It was amended subsequently, with additional categories of pesticides added under its umbrella in 1959, and the addition of cautionary words ("Warning," "Keep out of reach of children," etc.) and federal registration number to pesticide label requirements in 1964. FIFRA was substantially revised in 1972 under the Federal Environmental Pesticide Control Act (FEPCA, 1972), resulting in the addition of multiple provisions including mandating registration by the Environmental Protection Agency (EPA; created in 1970) of pesticides distributed or sold in the United States, ensuring that pesticides are distributed, sold, and used in such a way as to "not generally cause unreasonable adverse effects on the environment." Under FIFRA, the EPA must also act to prevent pesticide use from harming species listed under the federal Endangered Species Act (established in 1973).

Clean Water Act

The Federal Water Pollution Control Act of 1948, as amended in 1972, became commonly known as the Clean Water Act (CWA). This legislation established water quality goals for surface waters of the United States and controls discharge of pollutants, including pesticides, into water bodies. The CWA mandates that all point source discharges of pollution into the nation's waters require a permit, under the National Pollutant Discharge Elimination System (NPDES). Until recently, pesticides applied in or around water to control mosquitoes were exempt, under a 2006 rule titled "Application of Pesticides to Waters of the United States in Accordance with FIFRA," which clarified the EPA's interpretation that any pesticide registered under FIFRA for use around or in water that is applied according to FIFRA label restrictions is not considered a pollutant under the CWA and is thus not subject to NPDES permitting. This also held true for residues or degradates (breakdown products) of the pesticide.

However, in 2009 the Sixth Circuit Court determined that excess amounts of biological and chemical pesticides that enter water during and after application and their breakdown products are pollutants under federal law and so must be regulated under the CWA (National Cotton Council et al. v. EPA). Thus, application of FIFRA-approved pesticides in or around the waters of the United States now requires NPDES permitting. Specifically, an NPDES permit is required when an applicator will exceed a stated annual treatment threshold for discharge to waters of the United States of FIFRA-approved pesticides that leave a residue in four different use categories: control of mosquitoes and other flying pests; aquatic weed and algae control; aquatic nuisance animal control (invasive lamprey, zebra mussel, fish, nutria); and forest canopy pest control.

The overall goal of this ruling is to improve water quality and protect the health of the environment and of people by minimizing amounts of pesticide discharged. Opponents claim that FIFRA provides all necessary protections and that the new permitting requirements place an undue burden on applicators. Supporters of the ruling feel that the health-based standard (maximum contamination level) and provision of safest alternatives used by the CWA under their mandate of restoring and protecting our nation's waters minimize pesticide impacts and protect the environment better than FIFRA's more limited risk assessment. In addition, FIFRA is not sensitive to regional conditions or the needs of local waterbodies. The limits of FIFRA protections may be seen in the results of a nationwide survey conducted by the U.S. Geological Survey, which detected one or more pesticides or their degradates in every stream sampled (Gilliom et al. 2006), with half the streams sampled having concentrations of at least one pesticide that exceeded EPA guidelines for protecting aquatic life. The Sixth Circuit Court's ruling was implemented in October 2011. EPA estimates that over 350,000 applicators will be affected by this new rule; many mosquito control programs will need to assess ways to reduce their pesticide application levels, introduce or expand integrated management practices, and determine Best Management Practices (BMPs) for mosquito control and wetland management.

In March of 2011, the "Reducing Regulatory Burdens Act of 2011" (H.R. 872) was introduced in the U.S. Congress in an attempt to reverse the Sixth Circuit Court decision, and passed the House of Representatives in April 2011. The American Mosquito Control Association expressed official support of H.R. 872, stating that "These unnecessary permits will provide impetus for anti-pesticide activists to initiate legal challenges" (AMCA 2011) without protecting the environment, and maintaining that mandatory annual pesticide use reporting "will no doubt be used by activists, health scam and fraud perpetrators to leverage injunctive relief from applicators" (AMCA n.d.). Multiple agricultural and agribusiness organizations also supported H.R. 872. However, similar legislation introduced into the Senate failed, as did an attempt to insert the legislation into the Senate Farm Bill in 2012. In January 2013, a new bill (S. 175, 113th Congress) with the same goal of defeating the newly implemented NPDES requirements was introduced into the Senate and referred to the Senate Agriculture, Nutrition, and Forestry Committee, proposing that no additional permits be required to apply any pesticides registered under FIFRA.

Xerces and other environmental organizations oppose this bill and support the new NPDES permitting rules. The pesticides in question that are being applied in and around water have, of course, been approved by EPA under FIFRA, but FIFRA does not specifically protect water quality as does the CWA, which was enacted specifically to protect water quality. FIFRA approval involves the conclusion that use of the pesticide "will not generally cause unreasonable adverse effects on the environment," but multiple, well-documented incidents with pesticide-contaminated waters in the United States indicate that FIFRA label requirements are not sufficient to protect our waterways. More than 1,900 waterways in the United States are known to be impaired because of pesticides and many more that have not been sampled may also be polluted (U.S. EPA. Causes of Impairment for 303(d) Listed Waters Table, http://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T#causes_303d). The EPA's proposed general pesticide permit provides increased protection for rivers, streams, and wetlands because it would require pesticide applicators to analyze safer alternatives and monitor for environmental impacts post-application, thereby helping to ensure the safety of humans as well as the environment, and creating greater consistency within the community of pesticide applicators.

State and Regional Regulation

Establishment, organization, implementation, and funding of mosquito control agencies differs from state to state. In some cases, a state-level entity may be tasked with mosquito control; for example, the Delaware Code Relating to Mosquito Control has established the Department of Natural Resources and Environmental Control as the state's primary mosquito control entity (State of Delaware n.d.). In other cases, regional and local entities (often at a county level) are responsible, with states assuming an active role only in cases of emergency if local resources are inadequate.

Most mosquito control occurs locally through entities created at the city or county level. Many states have established statutes that allow for establishment of voter-approved mosquito abatement districts, which may operate at the level of a single city, all or part of a single county, or in multiple counties. Statues to establish mosquito abatement districts often include additional information such as specific functions of the district, enforcement authority, and funding mechanisms. Funding sources are varied and may include support from the state itself, special voter-approved taxation districts (mill levies), county or city general funds, surcharges added to utility bills, local sales taxes, private grants, and fee-for-service reimbursements. The Mosquito Abatement for Safety and Health Act (MASH Act, 108th Congress, Public Law 108-75), was passed in 2003 to enable the Centers for Disease Control and Prevention (CDC) to authorize grants to state and local governments to assist mosquito control programs, but this has not been reliably funded. The CDC may also provide funding in emergency situations when a disease epidemic occurs.

Depending on funding resources and perceived need, these mosquito abatement districts and associated vector control agencies may operate continuously or only during periods when public health concerns and/or mosquito-transmitted disease incidences are high. Decisions about local mosquito control programs may be made by city council members and county commissioners, who often consult further with public health departments, epidemiologists, and entomologists. Because local mosquito control methods can differ from those used on federal lands (fish and wildlife refuges, Department of Defense lands) as well as in state, county, and local parks and natural areas located within a mosquito abatement district, staff of these respective agencies may find themselves in continuing conflict as to the necessity and means of controlling mosquitoes. To find out more about the situation in your area, contact your county or city public health department.

4 Mosquito Control Methods

Chemical Controls

Organophosphates

PROS: more effective in polluted water than bio-controls such as Bti; if exposed to sunlight, breaks down fairly rapidly in the environment compared to other pesticides (unless bound to soil or sediment).

CONS: highly toxic to insects including beneficial insects such as bees; moderately toxic to fish; highly to acutely toxic to aquatic invertebrates; moderately to acutely toxic to vertebrates; possibility of resistance developing in target populations; can bind strongly to soils and sediments which increases persistence in environment; adulticide use provides much less effective control than reducing larval abundance; multiple applications per season permitted.

Organophosphate pesticides (OPs), derived from phosphoric acid, are active against a broad spectrum of invertebrates. They interfere with the action of enzymes called cholinesterases (ChE) that regulate the neurotransmitter acetylcholine (Ach), leading to muscular twitching, paralysis, and death (Ware 2004). Because ChE and Ach are also part of the vertebrate nervous system, OPs are highly to moderately toxic to vertebrates, although they generally degrade quickly under environmental conditions. Malathion and naled (registered for use in the United States since 1956 and 1959, respectively) are adulticides; temephos (registered in 1965) is a larvicide. Temephos (Abate) may be used in rotation with microbial pesticides or Insect Growth Regulators (IGRs) to delay resistance development (Floore 2006).

Pyrethroids

PROS: relatively low cost; good efficacy; low incidence of resistance in the field; lower toxicity to mammals and birds compared to other chemicals such as OPs.

CONS: toxic to aquatic invertebrates, crustacea, fish, and beneficial insects such as bees; piperonyl butoxide synergist commonly present in formulations is moderately to highly toxic to fish, amphibians, and other aquatic organisms, and is a possible human carcinogen; long half-life and persistence in soil and sediment; adulticide use provides much less effective control than reducing larval abundance.

Pyrethroids are synthetic forms of the pyrethrin pesticides derived naturally from chrysanthemum flowers. They affect the insect nervous system and have a rapid "knock-down" effect, but are generally used with a synergist such as piperonyl butoxide (PBO) that prevents the insect's system from detoxifying the pyrethroid, rendering it more effective. Because they act on the insect nervous system via a different pathway then OPs, they generally have low mammalian and bird toxicity, but are toxic to fish and tadpoles (NPTN 1998; EPA 2009). Permethrin, registered by the EPA in 1979, is the most widely used mosquito adulticide in the United States; of the estimated 32–39 million acres treated annually with adulticides, 9–10 million acres are treated with permethrin (EPA 2009).

Surface Oils and Films

PROS: effective control of mosquito pupae and newly-emerged adults.

CONS: toxic to surface-breathing aquatic insects, many of which are predators of mosquito larvae; negative effects on bird eggs and ducklings; creates undesirable sheen across water.

Surface oils (Golden Bear) and monomolecular films (Agnique, Arosurf) form a thin layer that reduces the surface tension of water and essentially causes mosquitoes to drown, as larvae, pupae, and adults are unable to attach to the water's surface to breathe, emerge, or lay eggs (Floore 2006). The surface oils last for a short time (~12 hours), but the monomolecular films may persist for up to two weeks. Sustained winds over 10 mph, runoff, rain, or tidal action displace films and result in poor mosquito control; the presence of vegetation or floating debris can also interfere with surface layer formation.

Biological Controls

Insect Growth Regulators

PROS: specific to arthropods; low mammalian toxicity.

CONS: toxic to aquatic insects and crustaceans; possible impacts on amphibians; some resistance to methoprene observed; affects all invertebrates that have a chitinous exoskeleton, so many different types of nontarget organisms affected.

Methoprene, a terpenoid compound that mimics the naturally-occurring insect juvenile hormone (JH) that controls insect development and maturation (Wright 1976), acts as a mosquito larvicide and pupacide. When JH levels are high, an insect molts from one juvenile stage (instar) to another. The drop in JH levels that occurs naturally triggers insects to molt from a juvenile to pupa or adult stage. If JH levels remain high, adult development cannot occur, and mortality is induced during molts to the pupal stage (reviewed in Henrick 2007). The presence of methoprene in the environment maintains high levels of a JH-like compound and suppresses development of adult characteristics. For insects such as mosquitoes and midges, which have complete development (larvae to pupa to adult), this results in decreased rates of pupation and adults that either fail to emerge or emerge with severe morphological abnormalities. Although methoprene was approved as a larvicide by the EPA in 1975 and has been in use for over 30 years, few cases of mosquito resistance in the field have been noted (Dame et al. 1998; Cornel et al. 2002).

The chemical diflubenzuron [1-(4-chlorophenyl) -3-(2, 6-difluorobenzoyl)-urea] acts as an IGR by interfering with the synthesis of chitin, the structural component of the exoskeleton of insects and other arthropods. Diflubenzuron acts as a larvicide and pupacide; when an insect attempts to molt from one stage to another, the inhibition of chitin synthesis results in death. Because all arthropods have chitinous exoskeletons, this is a broad-spectrum pesticide that affects insects, spiders, mites, zooplankton, and crustaceans (reviewed in Eisler 1992). Diflubenzuron was first registered for use in the United States against gypsy moth in 1979, but was subsequently approved for additional pest insects, including mosquitoes, by 1989 (Eisler 1992; EPA 1997).

Bacillus thuringiensis var. *israelensis*

PROS: acts specifically and rapidly against the lower Diptera (Nematocera), especially early instars (i.e., younger larvae), so direct toxicity to non-target organisms is confined to a smaller group of taxa; multiple different proteins comprising toxin decreases risk of resistance developing in target populations.

CONS: decreased efficacy against older, larger (i.e., later instar) mosquito larvae; decreased efficacy in polluted water; toxic to multiple groups of aquatic Diptera important in food web; repeated applications required to control mosquito larvae will persistently reduce or eliminate Nematocera in aquatic and terrestrial food webs.

Larvae of many species of mosquito, including those in the genus *Culex*, use a siphon tube to breathe at the surface of the water. (Photograph: Wikimedia Commons; James Gathany, CDC.)

Bacillus thuringiensis var. *israelensis* (Bti) is a naturally-occurring bacterium first isolated in 1976 (Goldberg & Margalit 1977). It was approved as a mosquito larvicide by the EPA in 1983 (EPA 1998) and is widely used in mosquito control (see review in Lacey 2007). When these bacteria undergo spore production (sporulation), they also produce an accompanying structure (parasporal body) comprised of multiple different proteins (Cyt1A, Cry11A, Cry4A, Cry4B) (Ibarra & Federici 1986). These proteins are inert until they are ingested by mosquito larvae and solubilized by the high (alkaline) pH of the larval midgut; the activated toxins disrupt the midgut cells and cause cessation of feeding and death. Bti is more specific than traditional chemical pesticides in that it does not affect all insects, but it is active against multiple types of Diptera (true flies), especially those in the suborder Nematocera, or lower Diptera. Within this suborder, the families Culicidae (mosquitoes), Simuliidae (black flies), and Chironomidae (non-biting midges) are the most susceptible, and Bti has been used extensively in the United States to control members of all three families. Efficacy of Bti can be strongly dependent on mosquito species, larval instar, larval density, temperature, and amount of organic material and vegetation in the habitat (reviewed in Boisvert & Boisvert 2000; Lacey 2007).

Bacillus sphaericus

PROS: specific to mosquitoes; more effective in polluted water than Bti; may recycle in environment.

CONS: more limited efficacy against some types of mosquitoes compared to Bti; some toxicity to crustacea; toxicity via a single protein increases the potential for resistance development.

Bacillus sphaericus (Bs), approved as a larvicide in 1991, is another spore-producing bacterium that produces a protein toxic to feeding mosquito larvae. Unlike Bti, Bs produces a single binary insecticidal protein (Charles et al. 1996); the presence of only one insecticidal protein is thought to account for observations of resistance to Bs (Rodcharoen & Mulla 1994; Rodcharoen & Mulla 1996, reviewed in Lacey 2007), as well as for its more limited spectrum of efficacy (Federici et al. 2003). Bs is active only against Culicidae (mosquitoes), but different genera within this family have different sensitivities; *Culex*, *Psorophora*, and some *Anopheles* species are the most sensitive, while *Aedes* are relatively insensitive (Lacey & Siegel 2000; Federici et al. 2003; Lacey & Merritt 2004; Lacey 2007). This bacterium also exerts sublethal effects on mosquitoes such as delayed pupation and emergence of adults with lower nutrient reserves and reduced survival rates (Lacey et al. 1987). There are some indications that it can recycle in the environment via spore production (Floore 2006). It is often the preferred larvicide in polluted waters, and may be used in alternating applications with Bti to help slow development of resistance (Zahiri & Mulla 2003).

Entomopathogenic Fungi

PROS: specific to mosquitoes; may recycle in environment.

CONS: difficult to mass produce; short shelf life; variable efficacy.

Fungal disease agents such as *Lagenidium giganteum* have been investigated in lab settings but have limited use and efficacy in biological control of mosquito larvae (reviewed in Lacey & Orr 1994; Scholte et al. 2004). *Lagenidium* has a novel life cycle in which infectious zoospores actively swim towards potential hosts. After coming into contact with the host, the spores produce growth that penetrates the larva's cuticle and eventually fills up the body space, killing the mosquito. New zoospores are formed on the surface of the cadaver and the cycle starts again. The fungus also produces desiccation-resistant oospores, which can persist in the environment for several years. However, the promise of *L. giganteum* as a biocontrol agent has been offset by the difficultly and expense of culturing the fungus on artificial media, short shelf life of infective zoospores, and environmental restrictions, as it is relatively ineffective in saline or polluted waters and at water temperatures outside of an optimal 15–35ºC (59–95ºF) range.

Gambusia (Mosquitofish)

PROS: easy to rear and transport; rapid reproductive rate; voracious predators

CONS: generalist feeders not specific to mosquito larvae; invade waterways and have damaging effects on aquatic ecosystems and native wildlife; variable efficacy, since mosquito abundances may increase as *Gambusia* consume aquatic invertebrates that are natural enemies of mosquitoes.

Gambusia affinis and *G. holbrooki* are often called mosquitofish, although *Gambusia* is a generalist predator and does not focus on mosquito larvae. Both species are native to the southeastern United States but have been distributed throughout much of the world as a mosquito control agent due to their ease of rearing and handling, voracious appetite, prolific reproduction rate, and tolerance for a wide variety of environmental conditions (Garcia 1983; Walton 2007; Pyke 2008). Characteristics that make *Gambusia* a desirable biocontrol agent, such as rapid reproduction and generally ravenous feeding, have had negative impacts on native fish and other wetland fauna in many places where it has been introduced (Garcia 1983; Rupp 1996). *Gambusia* do not control all types of mosquitoes in all habitats, as they feed at thewater's surface, are inefficient hunters in dense vegetation, and may even cause an increase in mosquito numbers as they consume predacious aquatic insects that feed on mosquito larvae. Mosquitofish are more effective at controlling permanent-water mosquitoes than floodwater mosquitoes, more effective against larvae of surface-breathing species, and perform better in habitats that lack vegetation (Meisch 1985). Studies done in the 1920s indicated that in many cases native fish were a better option for mosquito control (International Health Board 1924; Pyke 2008), although this work had little effect as *Gambusia* were already established as a mosquito control agent. In recent years the ability of native fish species to control mosquito abundance has received more attention (Walters & Legner 1980; Ahmed et al. 1988; Van Dam & Walton 2007; Pyke 2008; Irwin & Paskewitz 2009). *Gambusia* are still widely used and frequently distributed by mosquito control agencies to homeowners upon request.

Genetically Modified Mosquitoes

PROS: specific to vector species; does not involve application of pesticides.

CONS: mixed results; few field trials; unknown ecological and epidemiological effects; may cause permanent changes in mosquito populations that cannot be remediated; possibility that some transgenic mosquitoes may survive to adulthood in waters contaminated with low levels of tetracycline.

The possibility of creating genetically modified (transgenic) mosquitoes as a tool for vector control has been investigated for many years, but has had limited practical application and success. The genetic systems targeted usually involve either the ability of the female mosquito to act as a disease vector, or use sterile insect technique (SIT), in which the creation and release of sterile males reduces fecundity in local populations, as females mate with the sterile males and are then unable to lay fertile eggs (Crampton et al. 1990; Benedict & Robinson 2003; Franz et al. 2006; Raghavendra et al. 2011). As with any transgenic system, debate over release of genetically modified mosquitoes into the environment focuses on more than just efficacy, as concerns are raised about risks such as unanticipated genetic changes in wild mosquito populations, epidemiological changes in the diseases they vector, and potential expansion of an even worse vector to fill the void left by an extirpated species (Spielman 1994; Enserink 2002).

The issue of releasing transgenic mosquitoes became more immediate in recent years, due to the British company Oxitec conducting multiple large-scale field trials of their genetically engineered *Aedes aegypti* in an attempt to control dengue outbreaks. Using an approach called RIDL (Release of Insects carrying a Dominant Lethal), a constructed conditional lethal gene system is introduced into the mosquito germline. "Conditional lethal" means that expression of the lethal gene is suppressed when larvae are reared under certain conditions. In this case, the antibiotic tetracycline acts to suppress expression of the introduced lethal gene; when transgenic larval mosquitoes are reared in water containing tetracycline the gene is not expressed and they can grow to adulthood, but in the absence of tetracycline the gene is expressed and the transgenic larvae die.

Oxitec's field trials involve releasing lab-reared transgenic male mosquitoes at a high enough abundance so that local wild males are greatly outnumbered. When transgenic males mate with wild females, the resulting offspring die, as they carry the male's conditional lethal gene system and are not being reared with tetracycline. Their first field trial, conducted in the Cayman Islands in 2009, resulted in up to 80% reduction of wild mosquito numbers (Harris et al. 2011), but the company was criticized for not conducting controlled caged field trials first, and for lack of transparency and inadequate communication with the local citizens (Subbaraman 2011). No field trials have been conducted yet in the United States, although release of Oxitec mosquitoes was proposed in the Florida Keys. In 2009, the first outbreak of dengue in the Keys since 1934 occurred, with 93 cases reported. Intensive public education campaigns regarding larval source reduction around the home were implemented, and no new cases of dengue have been seen in the Keys since November 2010. However, with the possibility of future dengue outbreaks, Oxitec began working with local vector control agencies to test their genetically engineered mosquitoes in field trials in 2012. Public outcry, combined with the need for a detailed risk analysis by the U.S. Food and Drug Administration, delayed the Florida trials, but Oxitec was able to make another mass release in Itaberaba, Brazil in 2012 to control an outbreak of dengue.

Table 1. Pesticides Used for Mosquito Control in the Unites States.

Type	Pesticide	Trade names	Mosquito stage targeted	Typical maximum approved rate for mosquito control[1,2]	Nontarget aquatic organisms impacted
Organophosphates	Chlorpyrifos	Dursban, Mosquito-Mist, Pyrofos	Adult	Varies with formulation: 1–10 fluid oz./min. 0.005–0.01 lb a.i./ac. (fogging)	Moderately to very highly toxic to aquatic invertebrates, fish, and waterfowl.
Organophosphates	Malathion	Fyfanon	Adult	0.11–0.23 lb a.i./ac.	Very highly to highly toxic to freshwater fish, and freshwater and estuarine/marine invertebrates.
Organophosphates	Naled	Dibrom, Trumpet	Adult	0.02–0.1 lb a.i./ac.	Highly toxic to aquatic invertebrates and fish.
Organophosphates	Temephos	Abate	Larva	Varies with formulation: 0.05–0.5 lb a.i./ac. (granular) 0.016–0.048 lb a.i./ac. (liquid) 0.16–0.4 lb a.i./ac. (pellet)	Toxic to aquatic invertebrates and fish.
Pyrethroid	Permethrin	Ambush, Aqua-Reslin, Biomist, Permanone, Pounce	Adult	0.007 lb a.i./ac.	Highly toxic to fish and aquatic invertebrates.
Pyrethroid	Resmethrin	Scourge	Adult	0.0035–0.007 lb a.i./ac.	Highly toxic to freshwater and estuarine fish and invertebrates.
Pyrethroid	Sumithrin (d-phenothrin)	Anvil	Adult	0.0036–0.007 lb a.i./ac.	Very highly toxic to freshwater and estuarine invertebrates. Highly toxic to freshwater and estuarine fish. Toxic to amphibians.
Pyrethrum derivatives	Pyrethrins	Pyrenone, Pyrocide	Adult	0.008 lb a.i./ac.	Highly toxic to fish and aquatic invertebrates.
Surface oils and films	Petroleum-based mineral oils	Bonide, BVA, GB-1111, Golden Bear	Larva, pupa	3–5 gal/ac.	Toxic to fish and surface-breathing insects (corixids, belostomatids, dytiscids, notonectids).
Surface oils and films	Monomolecular films	Agnique, Arosurf	Larva, pupa, emerging adult	0.2–1 gal/ac. (fresh and brackish water) 0.35–1 gal/ac. (polluted water)	Toxic to surface-breathing insects (corixids, belostomatids, dytiscids, notonectids).
Insect growth regulator (chitin synthesis inhibitor)	Diflubenzuron	Dimilin	Larva/pupa	0.05 lb a.i./ac. (broadcast) 0.025–0.04 lb a.i./ac. (flooded pastures)	Toxic to marine and freshwater invertebrates.

(Continued on next page.)

Table 1 (continued). Pesticides Used for Mosquito Control in the Unites States.

Type	Pesticide	Trade names	Mosquito stage targeted	Typical maximum approved rate for mosquito control[1,2]	Nontarget aquatic organisms impacted
Insect growth regulator (juvenile hormone mimic)	Methoprene	Altosid	Larva/pupa	Varies with formulation: 0.007–0.013 lb a.i./ac. (liquid) 0.004–0.017 lb a.i./ac. (sand mix) 0.01–0.06 lb a.i./ac. (granular) 0.0058 lb a.i./ac. (briquettes) 0.014 lb a.i./ac. (extended release briquettes)	Very highly toxic to freshwater invertebrates. Slightly to very highly toxic to estuarine and marine invertebrates. Slightly to moderately toxic to freshwater fish.
Microbial (bacterium)	*Bacillus thuringiensis* var. *israelensis*	AquaBac, Bactimos, LarvX, Teknar, Vectobac	Larva	Varies with formulation: liquid = 4–16 oz./ac. (early instar larvae, clean water) to 16–32 oz./ac. (older larvae, polluted water) powder = 2–6 oz./ac. (clean water) to 12 oz./ac. (dirty water) corncob granules = 2.5–10 lb./ac. (clean water), 10–20 lb./ac. (polluted water) briquettes = 1/sq. ft. (clean water), up to 4/sq. ft. (dirty water)	Very toxic to Diptera (true flies), especially non-biting midges.
Microbial (bacterium)	*Bacillus sphaericus*	Spheratax, VectoLex	Larva	Varies with formulation: 5–20 lb/ac. (aerial or ground spray) 0.5–1.5 lb/ac. (granules)	No nontarget impacts noted.
Microbial (fungus)	*Lagenidium giganteum*	Laginex AS	Larva	9–180 fl. oz./ac.	No nontarget impacts noted.

1. Sources: National Pesticide Information Center (http://npic.orst.edu) Technical Fact Sheets and U. S. EPA Mosquito Control (http://www.epa.gov/mosquitocontrol/) and Reregistration Eligibility Decision (http://www.epa.gov/pesticides/reregistration/status.htm) Fact Sheets.

2. lb a.i./ac. = pounds of active ingredient per acre.

5 Impacts of Mosquito Control Agents on Nontarget Organisms

Direct Effects on Aquatic and Terrestrial Wildlife

Although many of today's commonly used mosquito control agents are more targeted and less toxic than those used in the past, their use still results in significant negative impacts to aquatic invertebrates in addition to mosquitoes (reviewed in Mulla et al. 1979). OPs such as temephos negatively impact a wide range of insects, and pyrethroids are highly toxic to many aquatic organisms (Hill 1989). IGRs such as methoprene and diflubenzuron affect insects and crustacea that share common pathways of hormone regulation and chitin synthesis. Bti is toxic to insect groups whose physiology is similar to mosquitoes, such as non-biting midges (chironomids) and other lower Diptera. Continued treatment of wetlands with these agents for mosquito control poses a risk to biodiversity, and resulting changes in invertebrate community composition can have widespread impacts on ecological interactions in the habitat.

Registration requires short-term acute toxicity tests to determine an LD_{50} (lethal dose at with 50% of the test subjects die). These tests expose the organisms in question to a range of concentrations of a single test compound in aqueous solution. However, tests are done on only a small suite of aquatic organisms, and they do not examine sublethal effects or the effects of chronic exposure to low doses of the compounds, nor do they examine the effects of other ingredients that are present in commercial formulations of the pesticide (many of which are considered proprietary by the pesticide companies and are protected as trade secrets) or potential interactions of multiple pesticides present in the environment (Clark 1991; Relyea 2009; Cothran et al. 2011). In the absence of direct tests for these variables, the EPA uses modeling techniques to predict outcomes based on data from acute toxicity tests (Hoff et al. 2010).

Furthermore, acute toxicity tests are necessarily done under conditions that are optimal for the test organisms apart from the presence of pesticides, and do not account for or reflect field conditions. Each pesticide is tested individually, and the synergistic effects of multiple pesticides likely to be in the environment simultaneously are not examined. Also, routes and durations of exposure in the field may vary from test conditions according to the nature of the pesticide used. For example, methoprene has been deemed unlikely to pose an unreasonable risk to nontarget aquatic invertebrates, in part due to its short half-life (rapid rate of degradation) in the environment. These conclusions are based on the results of short-term toxicity studies, however, and do not account for the potential impacts of repeated applications to maintain effective methoprene concentrations

Decades of widespread application of pesticides, insect growth regulators, surface oils, and bacteria have had extensive impacts on wetlands and the animal communities that rely on them. (Photo istockphoto/isgoodmyfrnds.)

in the habitat, or formulations developed to counter methoprene's short half-life such as slow release briquettes, pellets, or granules that may release methoprene over as much as 150 days.

The mosquito control agents described in the previous chapter are registered for use by the EPA (with the exception of genetically modified mosquitoes), whose registration process assumes that application of the control agent at the recommended label rate does not pose "an unreasonable risk" to nontarget organisms. In most cases these pesticides are not tested in real world conditions, so the actual negative impacts on nontarget organisms and on trophic function are unknown. Additionally, applications are made by humans, and errors such as accidental overspraying, spills, and unanticipated drift can and do occur. Field concentrations vary even when the recommended rates are applied, because water depths can change greatly within a single wetland, especially as a site dries down. In temporary wetlands in Minnesota that were treated with a 150-day slow-release methoprene formulation, methoprene concentrations in water samples taken from different areas within the site were highly variable (Hershey et al. 1995); half of the samples taken across the season had methoprene levels below detection limits, and the remainder had methoprene levels ranging from <2.5 μg/L (the concentration expected based on nominal daily release and average pond depth) up to 510 μg/L, which are well above recommended levels. In shallow stormwater catch basins, active ingredient from slow-release methoprene-based Altosid briquettes may be washed away during rainfall events and enter surface waters directly still bearing high concentrations of methoprene (Kuo et al. 2010). Temperature differences at different depths within aquatic habits can affect pesticide mixing, leading to differing concentrations in different parts of the habitat (Sudo et al. 2004; Jones et al. 2010; Cothran et al. 2011).

Unintentional or unanticipated drift from pesticide spraying can have severe negative impacts on the environment. The small droplets in ultra-low volume pesticide sprays that are needed to ensure effective coverage of a treated area can be affected in unanticipated ways by changes in wind, humidity, or temperature. Thus, although sensitive areas can be nominally protected by the establishment of no-spray zones, prevailing conditions at the time of spraying may result in unacceptable levels of contamination at these protected sites. A study in the Florida Keys found naled and fenthion residues downwind at up to 750 m or 50 m (respectively) in no-spray zones on wildlife refuges six hours after routine adulticiding (Hennessey et al. 1992). These no-spray zones were established because they harbored threatened or endangered species, including several butterflies and other pollinators, whose survival could be further threatened by unintentional drift.

Persistence in the environment will also differ based on the chemical nature of the pesticide. The chemical characteristics of pyrethroids allow them to remain adsorbed to aquatic sediments for much longer than the days to weeks that they persist in the water column (Laskowski 2002; Gan et al. 2005). Thus, an LD_{50} calculated for a species in aqueous solution will not account for field exposure via sediment in areas where repeated pyrethroid applications are made. A study in the highly agricultural Central Valley of California, where multiple pyrethroids including permethrin are used, indicated that sediment concentrations at which the aquatic invertebrate *Hyalella azteca* experienced impaired growth and death were only slightly above the analytical detection limit for pyrethroids in sediments (Amweg et al. 2005). Pyrethroid-contaminated sediments could thus provide a route of continued lethal exposure for nontarget invertebrates. Pyrethroid formulations also frequently contain the synergist piperonyl butoxide (PBO), which enhances the effects of pyrethroids by inactivating the insects' detoxifying enzymes. A study of the effects of a pesticide spraying program initiated in a metropolitan area in response to West Nile virus found that while the concentrations of pyrethroid and PBO applied did not appear directly harmful to aquatic life, increased sediment concentrations of PBO that occurred post-spray (2–4 μg/L) doubled the toxicity of pyrethroids already present in sediment due to general urban pesticide use (Weston et al. 2006). Changing conditions in the field such as temperature, food availability, predators, or dissolved oxygen levels create additional stressors that can also interact with the effects of pesticide exposure.

Nontarget Aquatic Invertebrates

Surface Oils and Films

Larvicidal oils and films form a thin barrier at the air-water interface and lower the water's surface tension. Aquatic invertebrates that obtain oxygen directly from the water via gills or diffusion across the integument appear to be unaffected by these materials. The monomolecular films Aerosurf and Agnique have little impact on organisms such as snails, crayfish, amphibians, fish, isopods, and amphipods, which do not depend on the air-water interface (reviewed in Nayar & Ali 2003). However, these films are lethal to atmosphere-breathing insects that rise to the surface to obtain oxygen, such as water boatmen, backswimmers, diving beetles, ostracods, copepods, and some mayfly nymphs (Mulla et al. 1983). Sentinel cages of water boatmen placed in ponds treated with Golden Bear oil showed almost complete mortality within 1–3 days of oil application to the site (Miles et al. 2002), and their numbers remained lower at treated sites compared to untreated for 3–15 days post-application.

Surface films used against mosquitoes can also dramatically reduce the number of chironomid midge larvae and pupae at a site (Mulla et al. 1983), which can have an impact on the aquatic food web (see "Indirect Effects" below). Because copepods, diving beetles, and backswimmers are major predators of mosquito larvae, these films can decrease the abundance of natural enemies and reduce overall mosquito control.

<u>Organophosphates</u>
Organophosphates are broad-spectrum insecticides that affect a wide range of invertebrates. Temephos treatment at 0.11 kg/ha or 28.01 kg/ha reduced chironomid midge larval abundance in a Delaware marsh by 20–200 fold (Laskowski et al. 1999), and treatment of a residential lake in Florida reduced chironomid abundance by 87–97% for over a month (Xue et al. 1993). Fenthion treatment of experimental ponds severely suppressed or completely eliminated some cladoceran and conchostracan crustaceans, as well as mayfly nymphs and water boatmen (Mulla et al. 1984a). In acute toxicity tests, the LD_{50} of temephos for freshwater copepods (0.0059 ppm) was lower than the LD_{50} for *Ae. albopictus* (0.0077 ppm) (Marten et al. 1993). A study investigating the hazards of mosquito control pesticides in national wildlife refuges in Delaware found significant reductions in overall diversity and abundance of aquatic invertebrates in ponds treated with field rates of temephos (Abate, 0.054 kg a.i./ha), with Ephemeroptera (mayflies), Odonata (dragonflies and damselflies), and Chironomidae (non-biting midges) severely impacted (Pinckney et al. 2000).

<u>Pyrethroids</u>
Pyrethroids can have significant impacts on nontarget aquatic organisms. Sensitivity to pyrethroids at levels close to those seen for mosquito larvae has been found in some mayflies, stoneflies, and caddisflies; water boatmen and backswimmers are also sensitive to a lesser extent (reviewed in Mian & Mulla 1992). Crustacea are particularly sensitive; in acute toxicity tests, LC_{50} values for pyrethroids were close to those seen for mosquito and black fly larvae (Mian & Mulla 1992). Studies done in Louisiana on the red swamp crayfish (*Procambarus clarkii*), which is cultivated for food production in the same areas where mosquito control is done, found an LC_{50} for the adulticide resmethrin of 0.00082 ppm (Holck & Meek 1987). This was several orders of magnitude lower than the LC_{50} values for three mosquito species tested in the same study (*An. quadrimaculatus*, *Psorophora columbiae*, and *Cx. salinarius* at 0.0023 ppm, 0.0056 ppm, and 0.012 ppm respectively). LC_{50} values for zooplankton such as *Daphnia* and *Ceriodaphnia* in 48-hour toxicity tests with permethrin and resmethrin were up to an order of magnitude lower than those seen for the mosquito *An. quadrimaculatus* (Milam et al. 2000).


Organophosphates disrupt the food web by harming many invertebrates, including dragonflies' aquatic nymphs. Both adults and nymphs are predators of mosquitoes. (Photograph: Celeste Mazzacano/The Xerces Society.)

<u>*Bacillus thuringiensis* var. *israelensis*</u>
Bacillus thuringiensis var. *israelensis* (Bti) is preferable to broad-spectrum insecticides due to its specificity for Diptera, and multiple studies have confirmed the lack of direct toxicity of label-rate applications to nontarget aquatic organisms other than true flies, such as crustaceans, water beetles, mayflies, caddisflies, dragonfly nymphs, and stoneflies (Mulla et al. 1982; Gibbs et al. 1986; Holck & Meek 1987; Roberts 1995; Gharib & Hilsenhoff 1988; Merritt et al. 1989; Molloy 1992; Wipfli et al. 1994a; Painter et al. 1996; Dritz et al. 2001; Eder & Schönbrunner 2010). However, its specificity against Diptera means that other true flies in addition to mosquitoes are affected. Bti is an effective control against black flies (Simuliidae) in flowing waters (Molloy 1990), and is also used to control non-biting midges (Chironomidae; Ali 1991). Mosquitoes and chironomid midges have similar enough physiology that Bti acts as a stomach poison in midges as well (Yiallouros et al. 1999). Some studies indicate that chironomids are less susceptible than mosquitoes to Bti and are not likely to be significantly affected by levels normally used in mosquito or black fly control (Ali et al. 1981; Yiallouros et al. 1999; Lundström, Schäfer et al. 2010a), but many others have shown that chironomids have equal or greater sensitivity, and the effects of Bti can vary depending on chironomid

genus or tribe (Ali 1981; Mulligan & Schaefer 1981; Back et al. 1985; Merritt et al. 1989; Mulla et al. 1990; Tozer & Garcia 1990; Rodcharoen et al. 1991; Molloy 1992; McCracken & Matthews 1997; Pont et al. 1999; Yiallouros et al. 1999; Ali et al. 2008).

Other nontarget Diptera that can be impacted by Bti treatment include Blephariceridae (net-winged midges), Ceratopogonidae (biting midges), Dixidae (dixid midges), Psychodidae (moth flies), and Tipulidae (crane flies) (Back et al. 1985, summarized in Boisvert & Boisvert 2000). In their review of 75 studies involving Bti products, Boisvert & Boisvert (2000) found that 37 indicated some impact of Bti on nontarget organisms. They also noted that much of the work done to examine the effects of Bti on target and nontarget organisms varied greatly in experimental design, methodology, and Bti formulation and dosage, to the extent that erroneous conclusions or conflicting results may have been generated.

<u>*Bacillus sphaericus*</u>

Bacillus sphaericus (Bs) is similar to Bti in its restricted host range and presumed lack of impact on nontarget organisms. Bs is preferable to Bti in some cases due to its lack of effect on Nematocera other than mosquitoes, greater effectiveness in polluted waters, and potential to recycle in the environment in mosquito cadavers (reviewed in Lacey 2007). Field studies have confirmed the lack of impact of Bs used in mosquito control on many nontarget organisms (Mulla et al. 1984a, 1984b; Merritt et al. 2005, reviewed in Lacey 2007), but Bs has measureable toxicity to some crustacea. Grass shrimp (*Palaemonetes pugio*) were sensitive to Bs spores, with a 96-hour LC_{50} of 39.25 mg/L (Key & Scott 1992). This falls within the range of application rates that may be used to control mosquitoes, as the LC_{50} for Bs varies among mosquito species and habitats. Mulla (1995) found a 48-hour LC_{50} of 0.044 mg/L for late instar *Cx. quinquefasciatus*, while *Ae. aegypti* larvae under the same conditions had an LC_{50} of 58.6 mg/L. However, mummichog fish (*Fundulus heteroclitus*) tested in the same experiment were resistant to Bs, with an LC_{50} an order of magnitude greater (Key & Scott 1992).

<u>*Gambusia*</u>

Part of the appeal of *Gambusia* fish as a mosquito control agent is their hardiness, rapid reproduction rate, and voracious appetite. However, as generalist predators, they feed on many different types of aquatic invertebrates, leading to variable outcomes in mosquito control (Ahmed et al. 1970; Hoy et al. 1972; Farley & Younce 1977; Hurlbert & Mulla 1981; Blaustein & Karban 1990; Blaustein 1992). Depending on the abundance and size (instar) of mosquito larvae and alternative prey in the habitat, *Gambusia* may preferentially feed on invertebrates other than mosquitoes. In some cases, mosquito numbers have actually become worse when *Gambusia* consumed all of the invertebrate predators of mosquitoes in the habitat (reviewed in Pyke 2008). In an experiment using both Bs and *Gambusia*, no adverse effects of Bs were seen on nontarget organisms, but *Gambusia* significantly decreased abundance of important mosquito predators including dytiscid beetles, phantom midge larvae, and backswimmers (Walton & Mulla 1991). Similarly, Lawler et al. (1999) found that outdoor ponds stocked with mosquito fish had significantly fewer dragonfly larvae and completely lacked backswimmers.

Insect growth regulators (IGRs) are often considered less harmful than chemical pesticides, but they have broad impacts on invertebrate communities. Crustaceans such as fiddler crabs are particularly suscetible to IGRs. (Photograph: Celeste Mazzacano/The Xerces Society.)

<u>Insect Growth Regulators</u>

Insect growth regulators (IGRs) are sometimes considered to have less effect on the food chain than other pesticides because they don't kill target insects immediately, allowing them to remain in the habitat for a longer time (Mulla 1991). However, both methoprene and diflubenzuron have significant toxicity to nontarget invertebrates and their use alters invertebrate community composition. Because of its broad activity against organisms with a chitinous exoskeleton, diflubenzuron application at rates that successfully suppress mosquitoes also impacts nontarget organisms such as chironomid midges, crustaceans (cladocera, copepods, amphipods, shrimp, crabs, crayfish), Hemiptera (water boatmen, backswimmers), and Coleoptera (predaceous diving beetles), as well as spiders, caddisfly larvae, dragonfly and damselfly nymphs, and mayfly nymphs (Miura & Takahashi 1974; Cunningham 1976; Christiansen et al. 1978; Farlow et al. 1978; Julin & Sanders 1978; Ali & Lord 1980; Rodrigues & Kaushik 1986; Wilson & Costlow 1986; Cunningham & Myers 1987; Yasuno & Satake 1990; Eisler 1992; O'Halloran et al. 1996). While some studies indicate that methoprene has little negative impact on nontarget aquatic invertebrates (Creekmur et al. 1981; Lawler et al. 2000, reviewed in Henrick 2007), many were conducted either over a short period of time or in habitats with limited diversity, and may not account for the effects of long-term chronic exposure from repeated site applications.

Significant acute toxicity and/or chronic effects of methoprene (such as developmental disorders, morphological defects, and reproductive anomalies) have been documented for a range of nontarget aquatic organisms including Diptera other than mosquitoes and midges, as well as various crustacean taxa, dragonfly larvae, and predaceous bugs and beetles (Norland & Mulla 1975; Gradoni et al. 1976; Mulla 1991; Gelbič et al. 1994; Chu et al. 1997; Glare & O'Callaghan 1999; Olmstead & Le-Blanc 2001; Cothran et al. 2011). Methoprene reduced or completely eliminated populations of non-biting midges in both laboratory and field studies (Miura & Takahashi 1974; Norland & Mulla 1975; Mulla et al. 1979, 1982; Norland & Mulla 1975; Creekmur et al. 1981; Yasuno & Satake 1990; Ali 1991, 1995a). Field investigations across eighteen months of aerial methoprene applications (28 g a.i./ha) in a Louisiana coastal marsh showed significant reductions in populations of fourteen aquatic taxa (Breaud et al. 1977), including several crustaceans (scuds, opossum shrimp, and freshwater shrimp), as well as specific taxa of aquatic insects such as dragonflies, damselflies, mayflies, water scavenger beetles, dance flies, and non-biting midges.

Crustacea are particularly susceptible to IGRs. Methoprene was implicated in an observed decrease in seasonal lobster (*Homarus americanus*) catches from Western Long Island Sound following pesticide applications to control West Nile virus vectors, as low levels of methoprene (starting at 1 ppb for young larvae) were subsequently found to have multiple adverse effects on larval and juvenile lobsters (Walker et al. 2005). Methoprene acted as a chemosterilant in male and female mud crabs (*Rhithropanopeus harrisii*) after 12–15 days of exposure to 1.39 ppm Altosid (Payen & Costlow 1977). Chronic methoprene exposure at levels representative of slow release formulations (0.1–1.0 μg/L) was linked to reduced post-molt weight gain and increased frequency of malformations during limb regeneration in male *Uca pugnax* fiddler crabs (Stueckle 2008). Methoprene application at standard rates used for mosquito control at a California wildlife refuge substantially slowed growth rates of cladocera, copepods, and ostracods (Meyer 1994). Chronic exposure to low concentrations of methoprene down to 0.2 nM significantly reduced growth rate and molting frequency of the cladoceran *Daphnia magna*, delayed reproductive maturation, and reduced brood size over multiple generations (Olmstead & Le-Blanc 2001). Lab studies on the acute toxicity of methoprene on a salt marsh copepod (*Apocyclops spartinus*) found that egg and early juvenile stages (nauplii) were sensitive to Altosid at concentrations of 0.8–2 ppm (Bircher & Ruber 1988), which the authors estimated to be an order of magnitude greater than the usual mosquito treatment concentrations in the field. Sublethal concentrations of methoprene interfered with larval development and metamorphosis of estuarine grass shrimp (*Palaemonetes pugio*) and mud crabs (*Rhithropanopeus harrisii*), and delayed brood production in mysids (*Americamysis bahia*) (McKenney 2005). The cladoceran *Moina macrocopa* had a relatively high LD_{50} for methoprene (0.34–0.51 mg/L) compared to field applications, but chronic exposure to methoprene doses 10-fold lower (0.05 mg/L), comparable to those seen in the environment after, reduced survival and fecundity (Chu et al. 1997). Similarly, Marten et al. (1993) found that for several freshwater copepods, the LD_{50} for Altosid was only 13 to 130 times higher than for *Aedes* mosquito larvae, and reproductive impairment was also seen.

Nontarget Terrestrial Insects

Insecticide sprays to control larval or adult mosquitoes can negatively impact the terrestrial insect community. General losses of biodiversity in insect communities that affect a wide range of orders and families have been noted by some researchers in areas where mosquito adulticides are sprayed (Emmel 1991; Kwan et al. 2009). Multiple studies have also shown negative impacts of mosquito treatments specifically on butterfly populations. Barrier treatments, in which pesticide applied as a ULV spray to foliage forms a coating or "barrier" that kills adults that come into contact with it, can have lethal and sublethal effects on adult or immature butterflies. Monarch butterfly (*Danaus plexippus*) caterpillars reared on milkweed leaves collected from areas where a routine permethrin barrier treatment was applied by mosquito control staff had significantly lower survival, even on leaves collected 21 days after permethrin treatment (Oberhauser et al. 2006). Development was also significantly slower in caterpillars reared on permethrin-treated milkweed plants in the laboratory. High mortality rates also occurred in monarch caterpillars and adults placed up to 120 m away from a resmethrin spray path (Oberhauser et al. 2009).

The decline of the federally endangered Schaus swallowtail butterfly (*Heraclides aristodemus ponceanus*), endemic to southern Florida, has been linked to pesticide applications for mosquito control (Emmel 1991; Eliazar & Emmel 1991). Populations of this butterfly on Key Largo appeared stable prior to 1972, at which time the mosquito control district switched from using malathion sprays to spraying Dibrom (naled) and Baytex (fenthion). Populations decreased sharply through 1985, recovered in areas where spraying was temporarily halted, then crashed again when spraying was resumed even though the larval host plant was abundant (Emmel 1991).

Mosquito control sprays are also recognized as contributing to the decline of the federally endangered Miami blue butterfly (*Cyclargus thomasi bethunebakeri*), a species endemic to southern Florida's coastal areas (Carroll & Loye 2006; FWS 2012). Larvae of the Miami blue have a mutualistic relationship with ants; caterpillars mature in the stems and seed pods of their host plant, and leave their entrance holes into the plant open so the ants that tend them can enter as well (Carroll & Loye 2006). In contrast, other species in this family (Lycaenidae) found in the Florida Keys do not have this relationship with ants, and they plug their entrance holes into the host plants to protect against predator entry. Roadside adulticide sprays may therefore have had a greater impact on the Miami Blue, as the unplugged holes in the plant allow greater penetration of pesticide; studies have found that both ants and Miami blue larvae died after mosquito spraying (FWS 2002; Carroll & Loye 2006). Trumpet EC ULV spray (0.75 oz/ha of 78% naled) applied a single time in north Key Largo to control *Ae. taeniorhynchus* mosquitoes significantly reduced survival of test populations of late-instar Miami blue butterfly larvae (Zhong et al. 2010). Naled is an OP that is highly toxic to invertebrates, but is thought to present a reduced threat due to its rapid degradation under normal environmental conditions. However, multiple sprays on consecutive nights are often done for mosquito control, increasing the length of exposure and risk of lethal or sublethal effects on butterfly larvae in an area.

The Schaus swallowtail was one of the first butterflies to be listed under the U.S. Endangered Species Act. Its continued decline has been linked to widespread use of mosquito-control pesticides in Florida. (Photograph: Bill Bouton.)

Population declines in other native butterflies with large historic distributions through southern Florida and the Keys such as Bartram's hairstreak (*Strymon acis bartrami*), Florida leafwing (*Anaea troglodyta floridalis*), and rockland skipper (*Hesperia meskei*) may also be linked to routine mosquito control spraying with the OPs naled and malathion and the pyrethroid permethrin (Salvato 2001). The apparent disappearance of a rare endemic taciturn wood cricket (*Gryllus cayensis*) from Key Largo in the 1970s was postulated to be linked to the advent of widespread fogging and ULV spraying for mosquitoes in the Keys, although this has not been proven (Walker 2001), and aerial mosquito spraying may also be a contributing factor in pollinator limitation in the Lower Florida Keys (Liu & Koptur 2003).

Fish

Temephos has significant negative impacts on nontarget fauna, including vertebrates. Juveniles of the crimson-spotted rainbowfish (*Melanotaenia duboulayi*), a native Australian species that is a mosquito predator with potential as a biocontrol agent, showed sensitivity to temephos at 40% of the Estimated Environmental Concentration (EEC; determined to be 67 ppb) from mosquito control uses (Brown et al. 2002). Earlier studies on a different species in this same genus showed even greater sensitivities to pyrethroids (Holdway et al. 1994). The mummichog (*Fundulus heteroclitus*), a dominant fish in estuarine tidal creeks on the Atlantic and Gulf coasts where a variety of pesticides may be used to control salt marsh mosquitoes, was sensitive to temephos in acute toxicity tests, with an LC_{50} of 0.04 mg/L; concentrations more than two-fold lower (0.018 mg/L) caused partial mortality and visible skin lesions (Lee & Scott 1989).

The mosquitofish *Gambusia* has negatively impacted some populations of native fish, and its history as a biocontrol agent has been punctuated by warnings from fish biologists about its impacts on ecosystems (reviewed in Rupp 1996). *Gambusia* introduction has been correlated with decreased abundance or local extirpation of a variety of native fish species via competition for habitat or food, or predation by *Gambusia* on the natives (Myers 1965; Schoenherr 1981; Blaustein 1991; Schaefer et al. 1994; Rupp 1996; Mills et al. 2004; Ayala et al. 2007).

Birds

Red-winged blackbird (*Agelais phoeniceus*) eggs exhibited decreased hatching success when treated with external applications of Golden Bear oil at three to ten times the amount expected to contact an egg in the field if the maximum recommended application rate of 5 gal/ac. is used (Albers et al. 2003). The authors concluded there should be no increased death of embryos when label rates are applied, but warned that possible effects on nestlings from additional pesticides received via aerosol sprays or from the feet and plumage of parent birds had not been investigated. Studies by Hoffmann et al. (2004) showed negative impacts of Golden Bear oil on mallard (*Anas platyrhynchos*) and bobwhite (*Colinus virginianus*) eggs at three to ten times the maximum label rate, including decreased hatching success and abnormal embryos and hatchlings, suggesting risks to birds under conditions of spray drift or overlapping applications. Ducklings held on ponds sprayed with Golden Bear oil at recommended rates exhibited matted feathers, continuous preening, and agitation, indicating that thermoregulation could be impaired in young birds, which places them at greater risk of hypothermia and decreases the amount of time spent foraging for food (Miles et al. 2002).

Howe et al. (1996) tested the effects of two ULV applications of malathion done five days apart on Brewer's sparrows (*Spizella breweri*) and sage thrashers (*Oreoscoptes montanus*) and found no significant direct effects on nest survivorship, although their results were variable across two years of study. However, they noted a significant reduction of the insect food base, and suggested that in years when food was not superabundant, survival of these insectivorous birds could be impacted following malathion treatments.

An additional direct impact of mosquitociding is exerted by the equipment used to treat sites. Aerial ULV sprays are delivered by low-flying aircraft, and their proximity to nesting birds can cause undue disturbance. Although the Migratory Bird Treaty Act of 1918 (16 U.S.C. §§ 703–712) protects migratory birds by prohibiting "take, possession, import, export, transport, selling, purchase, barter, or offering for sale, purchase or barter, of any migratory bird, their eggs, parts, and nests, except as authorized under a valid permit (50 CFR 21.11)", the definition of "take" does not include harassment. The Bald and Golden Eagle Protection Act (Eagle Act) enacted in 1940 (16 U.S.C. 668–668c) protects eagles from human activities that interfere with their ability to hunt, roost, nest, or reproduce, and includes a prohibition against disturbance. One of the eight categories of activities specified as likely to cause disturbance is "Helicopters and fixed-wing aircraft" (Category G). The U.S. Fish and Wildlife Service published additional National Bald Eagle Management Guidelines (FWS 2007) to help landowners and site managers meet the intent of the Eagle Act, and in some cases the Eagle Act may be used to designate areas where ULV sprays may not be administered. For example, when aerial mosquitocide treatments were proposed by vector control on an island in the Columbia River in Oregon that had active bald eagle nests, the U.S. Fish and Wildlife Service required a permit to be obtained for any activities undertaken during their nesting season (January 1–August 15), and a 1,000 foot setback was mandated (Dana Green, Natural Resources Manager/Aviation, Port of Portland, pers. comm., October 2012). Studies on raptors and waterbirds have found a variety of responses to aircraft overflights, ranging from no detectable response to wing flapping, temporary departure from an area, or complete escape and abandonment of nests (reviewed in NPS 1994). This can have negative impacts on breeding success, as eggs and chicks are vulnerable to temperature changes, loss of parental feeding activity, and predation when adults panic and take flight. Songbirds have been much less studied in this regard and their responses to overflight disturbance are not well known.

Studies show that tadpoles of the Pacific chorus frog are consumed by mosquitofish, even when mosquito larvae and other alternative prey are present. (Photograph: Celeste Mazzacano/The Xerces Society.)

Amphibians

Gambusia introduction has been linked with reduced amphibian abundance and diversity (reviewed in Pyke 2008). Laboratory and field studies have shown that *Gambusia* consume larvae or tadpoles of amphibians such as the California newt (*Taricha torosa*; Gamradt & Kats 1996) and Pacific chorus frogs (*Hyla regilla*; Goodsell & Kats 1999), even when mosquito larvae and other alternative prey are present. The decline of the California red-legged frog (*Rana aurora draytonii*) may be attributable in part to *Gambusia*. A study by Lawler et al. (1999) showed that while *Gambusia* did not decrease red-legged frog tadpole survival in outdoor ponds, tadpoles suffered a high proportion of injuries, had delayed metamorphosis, and weighed 35% less at metamorphosis.

The relationship between methoprene and observed malformations in field-collected amphibians continues to be debated. Methoprene and its breakdown products are structurally similar to retinoic acid, an important signaling molecule in vertebrate developmental pathways, and these substances can stimulate gene expression via the retinoic acid pathway in cultured vertebrate cells (Harmon et al. 1995). A study by La Clair et al. (1998) suggested that methoprene and its breakdown products caused significant malformations in the African clawed frog (*Xenopus laevis*). These results were seen at methoprene concentrations much higher (1 µL/L) than those expected in the environment when a normal application rate is used (0.004–0.006 µL/L), but the authors also cautioned about the effects of higher environmental concentrations resulting from multiple treatments or slow-release formulations, and synergistic effects with other environmental stressors. Sparling (2000) found greater frequency of limb malformations in southern leopard frogs collected from methoprene-treated wetlands (32 µg a.i./L) compared to those collected from control wetlands, but noted that malformations are also seen in frogs collected from areas with no known history of methoprene application. Additional studies offer conflicting conclusions: that methoprene causes malformations only when present at very high concentrations, that other stressors such as trematode parasites or ultraviolet light may be implicated, or that methoprene is just an additional stressor on a group whose health is already taxed by environmental contaminants and pathogens (Sessions & Ruth 1990; Ankley et al. 1998; Sessions et al. 1999; Degitz et al. 2003). The effects of environmentally relevant concentrations of methoprene on amphibians thus have yet to be clearly resolved, but warrant continued investigation.

Indirect Effects: Ecotoxicology, Community Interactions, and the Food Web

Few studies consider secondary effects and population-level responses in nontarget organisms resulting from chronic use or continued presence of mosquito control agents in the environment. Such responses include reduced feeding, changes in activity levels, altered mate-seeking or predator avoidance behaviors, reduced growth rate, decreased size at adulthood, and reduced fertility or fecundity. While many field tests are done with the laudable goal of determining the effects of mosquito control pesticides on nontarget organisms in a real-world situation, limitations in time and funding result in most field studies following the same pattern of acute toxicity testing done in the lab. Although the pesticides used may be applied at recommended label rates, these trials are often done over a short period, examining the effects of one or a limited number of field applications on a small suite of organisms (i.e., Jensen et al. 1999). Results of field trials can vary greatly depending upon factors such as the pesticide or combination of pesticides used, frequency and concentration of treatments, habitat type, environmental factors, and the particular nontarget taxa chosen for study (Lacey & Merritt 2004). Most do not accurately reflect the frequency and timing of pesticide applications done by vector control agencies, and thus cannot truly assess the risk of repeated applications done across multiple years. Ecotoxicology differs from traditional toxicology in that it examines the effects of pesticides on multiple interacting species under natural conditions (Relyea & Hoverman 2006), including synergistic effects between pesticides and other abiotic and biotic stressors in the environment. Also, instead of considering only acute toxicity, ecotoxicology models attempt to account for the effects of sublethal and chronic pesticide exposures on ecosystems.

Ecotoxicology investigations explore the wider, more complex arrays of community interactions and relationships. The direct action of pesticides in local elimination of selected groups can have much larger indirect community-level consequences (Relyea & Hoverman 2006), but predicting or detecting these changes is difficult due to the multiple complex interactions within ecosystems, many of which may not even be known. For example, repeated adulticide spraying to control mosquitoes in California was thought to contribute to an outbreak of pine needle scale due to the concurrent elimination of natural enemies of the scale insect (Dahlsten et al. 1969). A similar situation arose in Tennessee when an outbreak of Kermes scale occurred as an indirect and unforeseen effect of community-wide mosquito spraying to combat West Nile virus that also killed the parasitoid wasps that normally controlled the scale populations (Hale 2003). In the Lower Florida Keys, decreased pollinator abundance due to repeated aerial and ground mosquitocide applications has been suggested as a contributing factor to the decline of the rare endemic plant big pine partridge pea (*Chamaecrista keyensis*) (Liu & Koptur 2003). The sublethal effects of pesticide-induced behavioral changes can be even more difficult to test for and detect, and the magnitude of the impacts may not be seen until multiple years of treatment have occurred. Nevertheless, such changes are real and can have deleterious impacts in wetland ecosystems and on the structure and fitness of associated biotic communities.

Importance of Food Web Effects

Larvae of mosquitoes and other Nematocera, especially chironomid midges, provide an enormous food base in aquatic systems. However, they are an extremely important component of freshwater habitats (Ferrington et al. 2008), and constitute an enormous proportion of the animal biomass in wetlands. Their abundance, ubiquity, and diversity make them a vital part of the food web, as both consumers and prey. They feed on a wide range of organic materials, including suspended or deposited detritus, algae, plants, fungal spores, and, in some cases, other chironomid larvae (Berg 1995). They serve in turn as rich, nutritious prey for a variety of aquatic and terrestrial invertebrates as well as fish, amphibians, bats, waterfowl, wading birds, and some passerine birds. Wetland use by resident wildlife and migratory birds is often associated with density of aquatic invertebrates (Ali 1991; Armitage 1995; Weber & Haig 1996), and high densities of chironomid larvae can be a factor in selection of brood-rearing sites by mallard hens (Talent et al. 1982). Thus, pesticide-mediated removal of mosquito and chironomid larvae, whether by chemical or biological agents, has serious implications for the food web.

Relative to mosquito control, an ecotoxicology approach considers the ecosystem-level effects of removing a large proportion of the wetland food base from the habitat. Such food web effects extend beyond the aquatic habitat, as winged adult forms of aquatic insects are a critical link in terrestrial food chains. Emergence of adult aquatic insects from streams can provide 25–100% of the energy or carbon resources for upland riparian consumers such as birds, bats, and lizards (Baxter et al. 2005), so declines in aquatic invertebrate populations due to cumulative impacts of ongoing pesticide treatments have serious implications for the energy budget of the entire upland system. Furthermore, depending on the chemicals used, aquatic invertebrates that develop in pesticide-treated waters can transport their accumulated contaminant load to upland predators (Walters et al. 2008).

Negative food web-related effects of mosquito control on nontarget vertebrate populations have been documented for multiple groups. Pyrethroids sprayed over canopies for forest pests in areas that included streams reduced growth rates in caged salmon and native trout due to concurrent mortality of the aquatic invertebrates used as food by these fish (Kingsbury & Kreutzweiser 1987a, 1987b). Because of their reliance upon aquatic invertebrates during breeding and migration, chronic reduction in food supply from repeated insecticide applications can have long-term impacts on waterfowl and water birds. Ducklings could be doubly impacted by the effects of a breeding mother with fewer nutrient resources during egg development combined with reduced food availability post-hatching (Brown et al. 1985). A study in North Dakota found mosquito larvae or pupae in the esophageal contents of 19% of blue-winged teal (*Anas discors*), 13% of northern shovelers (*Anas clypea-*

The importance of aquatic invertebrates to the broader environment is illustrated by this barn swallow, which has returned to its nest with a mayfly for its offspring. (Photograph: Alistair Fraser.)

An ample supply of invertebrate prey is important for the survival and growth of ducklings of dabbling ducks such as mallards. (Photograph: Celeste Mazzacano/The Xerces Society.)

ta), and 7% of gadwall (*Anas strepera*) examined, with female ducks consuming an average of 3–5 times more mosquitoes than males (Meyer & Swanson 1982). Dabbling ducks also rely heavily on an abundant invertebrate food base during the first several weeks of life (Sugden 1973; Reinecke 1979), and early duckling growth rate is positively related to density of aquatic invertebrates in the habitat (Street 1978; Hunter et al. 1984; Cox et al. 1998). Studies examining the effects of mosquito control agents on nontarget invertebrates such as midges often point out that populations recover within a few weeks after a single application, but depending on the timing of application(s), those weeks could represent a critical feeding window for organisms that rely on these insects as an abundant nutrient source.

Impacts of Chemical Pesticides

Continuous treatment of wetlands with commonly used mosquito control agents such as methoprene and malathion has been linked to disruption of local food webs. A study on the effects of methoprene (0.1 lb a.i./ac.) on nontarget organisms in California rice fields found decreased abundance of multiple predator taxa such as dragonflies, giant water bugs, and water scavenger beetles (Case & Washino 1978). These changes were not apparent until one week after treatment, suggesting the possibility of an indirect food web effect. Malathion at relatively low doses (0.13–0.46 mg/L) had a cascade of indirect effects on survival of leopard frog (*Rana pipiens*) tadpoles in constructed habitats consisting of a diversity of phytoplankton, periphyton, and twenty-seven animal species (Relyea & Hoverman 2008). The direct effects of malathion in decreasing zooplankton abundance released phytoplankton from their grazing pressure, causing a bloom that led to lower light levels and thus decreased the amount of periphyton, which slowed the growth of leopard frogs that use periphyton as a food source. In laboratory mesocosms constructed with organisms at three different feeding levels (producers, primary consumers, and secondary consumers), treatment with even lower concentrations of malathion (20 and 100 µg/L) killed cladocera, and the resulting decrease in herbivory from this primary consumer group led to a phytoplankton bloom (Cothran et al. 2011). The survival of another primary consumer in this experiment, frog tadpoles, was affected more indirectly, as the direct mortality of predatory dragonfly nymphs from malathion treatment as well as potential indirect effects of reduced dragonfly foraging behavior resulted in lower tadpole mortality.

Impacts of Biological Controls

Biological control agents such as Bti are often seen as benign due to their specificity for the lower Diptera (Nematocera). However, from a broader ecological standpoint, the ability of Bti to cause rapid, significant decreases in Nematocera has important implications for the aquatic food web and the community it shapes. Not only is the overall abundance of Nematocera larvae decreased by Bti, their relatively synchronous death following Bti treatment also puts a large pulse of detritus into the system, and this sudden transition affects organisms at different trophic levels. In assessing probable effects of black fly mortality on the food web in Michigan rivers following Bti treatment, Merritt et al. (1991) found that although a predaceous corydalid (hellgrammite; *Nigronia serricornis*) accepted live or Bti-killed larvae equally as a food source and would likely be unaffected following Bti treatment, a co-occurring predatory stonefly (*Acroneuria lycorias*) selected strongly for live prey, while a detritivorous stonefly (*Prostoia completa*) fed on dead black fly larvae. A similar study found that the caddisfly *Ceratopsyche sparna* fed much more readily on Bti-killed black fly larvae than on live larvae (Wipfli & Merritt 1994a). In contrast, the predatory stonefly nymph *A. lycorias* avoided Bti-killed black fly larvae and ingested significantly less prey overall following Bti treatment of a stream in which over 95% of the prey base consisted of Nematocera, and showed little ability to switch to alternative prey (Wipfli & Merritt 1994b). Elimination of Nematocera from a prey community following Bti treatment can thus have a range of impacts on the aquatic community, affecting feeding ability of generalist vs. specialist predators, and changing the abundance and composition of alternative prey as predators engage in prey-switching behaviors. Taxa with different feeding strategies (i.e., predators, detritivores, filter-feeders) could either completely lose their prey base or gain a huge biomass of food following Bti treatment, which would further affect their development and abundance and alter competitive relationships in the habitat.

Only a handful of studies have investigated long-term effects of continuing Bti-based mosquito control and accompanying removal of prey base on wetland invertebrate community composition in the field, and results have varied. No long-term changes in community composition of nontarget organisms were reported in conjunction with ongoing Bti-based mosquito control in Germany's Rhine Valley (Becker 1997). Temporary wetlands in Sweden treated with Bti across six years to control floodwater mosquitoes showed no significant impact on the overall production of chironomids (Lundström, Schäfer et al. 2010), but the chironomid species richness and turnover rate in species composition was significantly higher at treated sites (Lundström, Brodin et al. 2010), suggesting a disturbed system with higher random repeated colonization by different chironomid groups, some of which are then extirpated. Additional ecological impacts on chironomids of reduced competition from mosquito larvae were also postulated. Temporary wetlands in Minnesota treated regularly with field rates of Bti (Vectobac-G, 11.72 kg/ha) or methoprene (Altosid 3-week release granules, 5.82 kg/ha) had no significant nontarget effects after the first year of treatment, but insect species diversity and abundance were lower in years two and three (Hershey et al. 1998; Niemi et al. 1999). Much of this decrease was due to direct reduction of Nematocera, especially chironomids. However, by the third year of the study total predator density decreased, and numbers of predatory diving beetles (Dytiscidae) and water scavenger beetles (Hydrophilidae) were significantly lower, which suggests food web effects resulting from the continued local mortality of chironomids and mosquitoes. No concurrent decrease was observed in zooplankton populations, and the reproductive success of selected resident songbirds and waterfowl did not appear to be impacted (Hanowski et al. 1997a, 1997b, 1997c; Niemi et al. 1999), but the authors concluded that the birds likely completed their reproductive cycle in the window before mosquito control lowered aquatic insect abundances, and cautioned that decreased insect abundances later in summer could affect dispersal patterns and later survival of young birds as well as use of treated wetlands as stopover sites for migrating birds. In contrast, house martins (*Delichon urbicum*) in a region of France with ongoing Bti-based mosquito control exhibited diet alterations and reduced breeding success due to the trophic effects of Bti (Poulin et al. 2010). Intake of midges and mosquitoes as well as taxa that prey on them (spiders and dragonflies) was significantly reduced among martins at treated sites, and martin breeding success was linked to consumption of these groups.

Gambusia affinis has been used for years as a biological control agent; while native to the southeastern United States, this species may now be the most widely distributed freshwater fish in the world (Gerberich & Laird 1965; Pyke 2008). Although used successfully in many cases (Hoy et al. 1971, and reviewed in Pyke 2008), there are also many incidences cited in the literature where *Gambusia* either had no discernible effect on mosquito populations, or was associated with an increase in mosquito larvae (Ahmed et al. 1970; Hoy et al. 1972; Farley & Younce 1977; Hurlbert & Mulla 1981; Blaustein & Karban 1990; Blaustein 1992). The general and voracious feeding habits of *Gambusia* create a variety of complex direct and indirect ecological interactions that can lead to different outcomes in mosquito control. In California rice fields, *Gambusia* preyed on predatory bugs, beetles, and odonates (Bence 1982, 1988), thereby reducing overall predation pressure on mosquito larvae. *Gambusia* also preyed on zooplankton (copepods, ostracods, and cladocerans), an alternative prey for both other fish and insect predators, even when mosquito larvae were present; after zooplankton abundance decreased, the fish switched to the more-abundant mosquito larvae as a food resource (Bence 1988).

Gambusia introduction has been correlated with decreases in populations of native fish, amphibians, copepods, and highly endemic fairy shrimp (reviewed in Mulla et al. 1979; Pyke 2008). The virtual elimination of zooplankton by *Gambusia* in pond studies reduces biodiversity and restructures vernal pool communities (Gamradt & Kats 1996; Leyse et al. 2004), and is thought to have contributed significantly to the decline of the endangered Sonoran topminnow (*Poeciliopsis occidentalis*) (Meffe et al. 1983). *Gambusia* introduction has caused eutrophication of artificial ponds, as intense feeding by the fish on resident zooplankton release algae from the grazing pressure normally exerted by zooplankton and enable large algal blooms to develop (Hurlbert et al. 1972; Hurlbert & Mulla 1981). In addition, insects may be eliminated in pools containing *Gambusia*, while fishless pools in contrast are inhabited by large numbers of chironomid midges, small minnow mayflies (Ephemeroptera: Baetidae), and shore flies (Diptera: Ephydridae) (Hurlbert et al. 1972), or by tadpoles and predaceous backswimmer bugs (Hurlbert & Mulla 1982). The effects of *Gambusia* predation and the competition for resources engendered by their voracious appetites can thus alter the structure of aquatic ecosystems and remove important natural enemies of mosquito larvae.

6 The Human Element in Effective Mosquito Control

Public Education is the Key to Public Health

Public health takes precedence where mosquito control is concerned, but the response must be appropriate and effective. Mosquito-borne diseases such as St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), and Eastern Equine Encephalitis (EEE) have long been present in the United States. The advent of West Nile virus (WNV) in 1999 was a disturbing reminder of our continued vulnerability to insect-vectored disease, and many municipalities implemented broad-scale aerial adulticiding programs in response to the first disease incidences in human populations. However, a community-based mosquito control program founded in public education, community involvement, and data on mosquito populations, combined with scientifically based information on the best means of control, can achieve effective mosquito and disease control without the need to abandon IPM practices and engage in widespread spraying. The Mosquito Control Collaborative sponsored by the Centers for Disease Control stresses the vital role of public education in successful mosquito and disease control programs, stating that "Development of a communications plan that includes public education about preventing the breeding of mosquitoes, personal protection guidance, and the activities and success of the agencies involved is critical to the success of the program." (Mosquito Control Collaborative 2005).

Different mosquito species have been implicated as WNV vectors to differing degrees, but any incidence of disease often leads to wholesale mosquito control. Deaths from disease that can be avoided are tragic, but it should be noted that ~80% of those who become infected with WNV show no symptoms. Those that do become symptomatic generally experience mild flu-like illness; less than 1% of those infected develop a severe and sometimes fatal neuroinvasive disease (CDC 2012), with people over the age of 50 at higher risk for severe illness. Only a small number of our total mosquito species act as important bridge (bird-to-human) vectors, and the primary vector species can differ in different parts of the country. Furthermore, varying practices, attitudes, and conditions among different communities will affect their exposure to mosquitoes as well as the most effective types of control measures to be applied. Thus, even—or perhaps, especially—in a public health situation, knowledge of vector capacity and ecology of different species allows control to be targeted to the appropriate habitats and times, and widespread repeated aerial pesticide applications are neither necessary nor effective. For example, just two among ten mosquito species collected in New York state were responsible for up to 80% of the risk for human WNV infections in the area (Kilpatrick et al. 2005). These species (*Cx. pipiens* and *Cx. restuans*) are both container breeders, so treatment of all wetlands in the area would not be an optimal or cost-effective control plan. Control targeted to specific vector species habitats will reduce vector populations while minimizing impacts on nontarget wetlands and wildlife.

The Centers for Disease Control advocate WNV prevention based on community-level control programs to reduce mosquito larva sources, and increased personal protection to avoid being bitten (CDC 2012). The main WNV vectors are mosquitoes in the genus *Culex*, which breed in containers, often in polluted water or water with a high organic content, and do not tend to fly far from their emergence site. Increased aggressive mosquito control in the wake of WNV has heightened public concerns about both the disease and the effects of widespread adulticiding. The Maine Environmental Policy Institute published a report in which they contrasted the

Stagnant water in gardens: Public education is effective in removing this source of mosquitoes and associated biting. (Photograph: Matthew Shepherd/The Xerces Society.)

relative risks of WNV, with only 1 in 150 people bitten by an infective mosquito developing serious symptoms, with the even greater risks to human health and to the environment from pesticide applications. Several municipalities have rejected widespread adulticiding as a viable response to WNV. In 2000, the District of Columbia implemented a mosquito control program that did not use aerosolized adulticides, citing the region's high incidence of asthma as well as the potential for spray drift to affect nontarget organisms on federal lands (Hinson 2004), including rare aquatic invertebrates. Fort Worth, TX, discontinued spraying against nuisance-biting mosquitoes in 1991, citing the high incidences of asthma and allergies among residents, and did not resume spraying in 2003 after WNV appeared in the United States. They relied instead on educating citizens to reduce numbers of mosquito larvae by eliminating breeding sites and decreasing contact with adults via repellants and appropriate clothing. However, following a major flare-up of WNV in Texas in 2012 with several fatalities, Fort Worth instituted spraying operations, although they limited spraying to targeted zip codes and used ground foggers instead of aerial applications. The city of Shaker Heights, OH, developed a WNV response plan that relies heavily on surveillance, source reduction, and personal protection, with adulticiding considered only after confirmation of locally acquired human cases in targeted areas, if deemed effective (West Nile Virus Community Task Force of Shaker Heights, OH 2002). Lyndhurst, OH, soon followed, passing an ordinance prohibiting pesticide spraying for WNV in 2003 (Ordinance No. 2003-37), citing the risks of pesticide exposure along with the relative inefficacy of adulticiding and a lack of scientific data regarding reduced WNV incidence after spraying.

The City of Boulder, CO, had not controlled mosquitoes prior to the outbreak of WNV in 2003, as the effects of nuisance biting were not thought to outweigh the environmental impacts of pesticide application (City of Boulder 2006). The mosquito control plan developed in response to WNV focused on identifying areas where vector *Culex* mosquitoes (especially *Cx. tarsalis* and *Cx. pipiens*) were reproducing, thus avoiding the time and cost of treating larval habitats where nonvector, nuisance-biting, or nonanthropophilic mosquitoes were found, and of treating wetlands that were considered potential breeding sites but found to lack mosquitoes (OtterTail Environmental, Inc. 2003; City of Boulder 2006). A nuisance mosquito control program was implemented in 2007, following the same procedures and treating a limited number of targeted habitats with Bti when larval abundance of nuisance-biting species was high (OtterTail Environmental, Inc. 2012). The approach requires increased surveillance work and a team with greater entomology skills and GIS capability, but is cost-effective in that it results in less treatment overall (both larvicide and adulticide) of wetlands and other habitats. It also enables detailed larval habitat mapping, creating a base to be categorized and prioritized for surveillance and treatment in future years. Bti is the only larvicide used, based on its low persistence in the environment and likelihood of causing the least harm to treated habitats; it is applied at low rates and accompanied by post-treatment monitoring to ensure that treatment is effective. Informed decisions whether to spray with adulticides are based on adult surveillance and persistent WNV incidence, and considered only as a final option, in which case ground application of pyrethroids is done in targeted "spot treatment" areas (City of Boulder 2006).

In New York state, local authorities make decisions about mosquito control measures, including adulticiding. However, the state Department of Health mosquito-borne illness response plan (NYSDOH 2012), which covers both WNV and EEE responses, states: "Aerial adulticiding has uncertain and potentially, very limited benefits for preventing illness among humans…Given the limitations of adulticiding, the primary strategy to prevent mosquito-borne illness among humans must continue to be promotion of personal preventive measures." The report stresses the effective role the public plays in this process, and the fact that source reduction of container-breeding mosquitoes can remove the need to use pesticides in and around affected areas.

Public Education Changes Human Behavior and Disease Incidence

Personal protective behaviors are a key factor in preventing West Nile virus in humans (Campbell et al. 2002). Most WNV public education campaigns urge people to engage in locally appropriate variations on "the 4 Ds": DEET (use an insect repellant containing DEET); Dress (wear long sleeves and pants); Drain (remove standing water sources around the home); and Dusk and dawn (avoid being outside during these peak vector-mosquito biting periods).

The true measure of the success of public education lies in the extent to which citizens change their behaviors and engage in activities that affect disease epidemiology by reducing mosquito numbers and/or human contact. While linking education to behavioral outcomes can be challenging at many levels (see "Knowledge vs. Practice" below), public education campaigns have documented success. A survey in Oakville, Ontario, during a WNV outbreak found more than a 50% reduction in infection risk when two or more personal protection measures were reported as having been taken (Loeb et al. 2005). A serious outbreak of St. Louis Encephalitis (SLE) in central Florida in 1990 was countered in part by intensive

An example of multi-lingual posters (in English, Korean, Spanish) from Multnomah County Vector Control in Oregon.

public health education advising people to curtail their outdoor activities during the early morning and evening hours when the *Culex* vector is most active (Meehan et al. 2000). Community surveys found that people who were aware of personal protection measures advocated in the public health campaign and changed their behaviors accordingly were four times less likely to acquire SLE. Infection rates were highest among the homeless and in impoverished areas where houses were poorly tended and thus less likely to have window screens or air conditioning, and more likely to have neglected mosquito breeding sites, indicating that public authorities need to conduct additional work with homeless shelters and in areas of low socioeconomic conditions.

While a "sky is falling" approach to mosquito-borne disease should never be encouraged, people who are more concerned about disease will be motivated to take effective personal protection measures. A survey of two neighborhoods in Ithaca, NY, found a significant relationship between perceptions of WNV and decrease in standing water sources around the home (Tuiten et al. 2009). People who reported concern that WNV would harm a member of their family were six times less likely to have a container that harbored mosquito larvae in their yards. Interestingly, no significant relationship was seen between level of knowledge about WNV and the presence of mosquito-positive containers in respondents' yards. In a study done in two cities in Queensland, Australia, where mosquito-borne diseases such as dengue and Ross River virus are common, the efficacy of public education was demonstrated in surveys that showed almost all respondents knew that mosquito vectors breed in artificial containers, and over three-quarters reported that they actively prevented mosquitoes from breeding in their yards (Larson et al. 2000). Concern about mosquito-borne disease was found to be a significant predictor of removing larval breeding sources around the home and of using personal protection, even when responses indicated an imperfect understanding of mosquito ecology and disease transmission. Similar results were seen in a survey of British Columbia residents in which 73% of respondents indicated that concern about WNV prompted them to remove standing water around their homes (Aquino et al. 2004).

The importance of public campaigns urging personal protective measures was highlighted by a study done in the adjacent (and demographically similar) cities of Loveland and Fort Collins, CO (Gujral et al. 2007). Both experienced WNV outbreaks, but the incidence of severe disease was over twice as high in Loveland, which had a more extensive mosquito control program, fewer mosquitoes overall, and fewer WNV-infected mosquitoes than Fort Collins. However, Loveland residents were 39% more likely to seldom or never use repellants and 30% more likely to be outdoors during periods of high mosquito activity, compared to Fort Collins residents. Personal protective measures are thus shown to have an important impact on infection rates, even where mosquito control programs are active, especially as people may feel a false sense of security if they know mosquito control is done in their area, feeling that the responsibility of personal protection has thus been removed. Loveland had a long-standing mosquito control program prior to WNV, while Fort Collins instituted a new emergency control program in response to the WNV, so this perception may have been a factor.

Perceptions about Wetlands

Mosquito control is further complicated by the public's conflicting attitudes towards wetlands. Increased urban and suburban development brings more people into closer proximity with previously undeveloped sites where mosquitoes may breed, and residents can simultaneously express appreciation for aesthetic and environmental aspects of a nearby wetland in conjunction with a profound intolerance for mosquitoes. Residents of Columbia, MD, showed an overwhelmingly positive attitude about the ability of urban wetlands to add beauty, diversity, and quality to the human environment as well as to increase housing values, and thought stormwater control structures should also be managed as wildlife habitat (Adams et al. 1984). At the same time, insect problems were rated 2nd on a list of wetland nuisance values, suggesting a lack of connection between residents' appreciation of fish and waterfowl and their knowledge of the importance of aquatic invertebrates in sustaining these animals. Intensive applications of pesticides in and around the home may also make people who experience few to no mosquito bites at their backyard barbecues intolerant to any bites under the very different conditions at a wildlife refuge.

Citizens frequently express misconceptions about wetlands and mosquitoes, assuming that all wetlands produce mosquitoes, all mosquitoes are vectors of viruses that cause diseases such as West Nile virus or Eastern Equine Encephalitis, and that any level of mosquito biting is insupportable (Morris 1991). The impact of such misconceptions is exacerbated by the fact that pesticide treatments can be triggered by public calls made to a local mosquito control agency about nuisance biting. These misconceptions can only be alleviated by ongoing education and outreach programs. In Simpson, NC, where residents were concerned about mosquito impacts from a newly constructed wetland in their community, surveillance done in response to their concerns showed no significant differences in overall mosquito abundance in the area before and after wetland construction (Anderson et al. 2007). The results not only allayed public concerns about the wetland but also revealed other areas of intensive mosquito production around the city to be targeted for treatment. A survey of knowledge and attitudes about wetlands among inhabitants of the Stony Brook – Millstone watershed in New Jersey revealed that although citizens stated strong support for wetland conservation, they had limited understanding of what constituted a wetland and were unaware of both the functions and locations of wetlands within their own towns (Johnson & Pflugh 2008). Such a lack of understanding could impact people's ability to support or engage in activities that preserve wetlands, or to accurately evaluate wetland management practices or mosquito production potential.

Knowledge vs. Practice

Gaps in knowledge vs. practice can occur among both the public and vector control agencies. Best Management Practices (BMPs) for mosquito control developed around integrated management plans are often published at the state level by agencies such as health departments or mosquito control boards (New Jersey Department of Environmental Protection 1997; Washington State Department of Ecology 2004; Massachusetts State Reclamation & Mosquito Control Board 2008; Connelly & Carlson 2009; California DOH and MVC Association of California 2010). These BMPs are usually based on integrated management principles and recommend techniques that help reduce pesticide use and impacts. Regional mosquito control districts are encouraged to adopt these BMPs but actual local practices may vary widely and are affected by differences in funding, staffing, training, and entomological and biological expertise. Cities may thus be faced with a choice of either attempting to perform mosquito control independently with insufficient resources and capacity, or entering into a mosquito control district whose practices are seen as undesirable by the community. In Massachusetts, the state Audubon Society recommended that communities resolve this issue by requesting their local mosquito control district to enter into a binding agreement to provide services in accordance with the MA Department of Health integrated management plan for mosquito control (Mass Audubon 2012).

Not all vector control agencies have the staff, funding, entomological expertise, or resources to develop and implement a true integrated management program, and may thus fall back on scheduled treatments of standing water, which is contrary to the established tenets of integrated management. A survey done in Mississippi in conjunction with federal funds disbursed to help control mosquitoes in the aftermath of Hurricane Katrina showed that most municipal mosquito control consisted of routine spraying, based primarily on complaints from the public and the presence of standing water (Edwards et al. 2009). Only a small proportion of mosquito control personnel surveyed indicated that adult or larval surveillance was used to inform treatment decisions. While this is by no means the case for all mosquito control programs, this study points out that even after workshops emphasizing community education and integrated management were offered around the state prior to fund disbursement, and despite the fact that Mississippi had published BMPs for mosquito control (Goddard 2003), significant gaps in knowledge and practice persisted.

Citizens experiencing a mosquito problem are frequently completely unaware of their own role in harboring mosquitoes. Callers complaining about nuisance biting often blame the nearest wetland without realizing that their own yard (or those of their neighbors) has flights of mosquitoes emerging from stagnant birdbaths, pet bowls, unattended swimming pools, gutters, or plant pot saucers (Grodner et al. 2007). The Centers for Disease Control guidelines for West Nile virus control (CDC 2003) stress that education to inform the public about local mosquitoes and related insect-vectored disease issues and to promote adoption of preventive behaviors is key to a successful integrated management program. However, it also notes that simply providing information is generally unlikely to alter existing behaviors, and encourages developing local task forces, social marketing, and outcome evaluation strategies. McNaughton et al. (2010) found that while public outreach campaigns achieved a greater awareness of dengue among residents of Queensland, Australia, they did not achieve the additional desired goal of a greater understanding of the mosquito that vectors the disease. Residents surveyed assumed that *Ae. aegypti*, the peridomestic container-breeding mosquito that is an important dengue vector in Australia, was ubiquitous in the landscape and that most of its breeding sites were located beyond public spaces, and therefore beyond the control and responsibility of city dwellers. Thus, the critically important mosquito control activity of source reduction was not being done. Simply providing information, even repeatedly, is not necessarily sufficient to ensure increased understanding and alter behaviors, and an understanding of existing attitudes and potential barriers to understanding or changing behavior must also be developed.

Expansion of suburbs into the countryside has brought people into closer contact with wetlands, which they can be quick to blame when mosquitoes start biting. The source of mosquitoes, however, is often closer to home. (Photograph: Matthew Shepherd/The Xerces Society.)

A keystone of community-based vector control involves the public taking responsibility for removing mosquito breeding sources from their homes and yards, and implementing personal protection measures by wearing protective clothing, using repellants, and avoiding sitting outdoors during hours of peak mosquito activity. While information about the role of the public in mosquito management is often available to some extent from regional vector control or public health agencies, gaps between levels of success in educating the public and changing their behavior interferes with effective integrated management. A study done by health officials in Cambridge, MA, following the first outbreak of WNV found that most people were well-informed about ways to eliminate mosquito breeding sites, but over one-third had not taken measures to remove sources of standing water in their yards (reported in West Nile Virus Community Task Force of Shaker Heights OH 2002). In a study done in Ithaca, NY, 60% of survey respondents knew that removing standing water in their yards would eliminate mosquito breeding sites, but 38% of this group reported that they had not done so because it was too much work or too difficult to do (Tuiten et al. 2009). In such cases, public outreach may need to be accompanied by penalties for non-compliance; for example, the outreach and education program instituted by the District of Columbia Department of Health reached hundreds of thousands of people and was considered highly successful, but they also found it necessary to establish civil fines on individuals and businesses that did not engage in published mosquito source reduction practices as creating a "public health nuisance" (District of Columbia DOH 2004).

Public education campaigns have different levels of impact among different demographic groups, including gender, age, socioeconomic status, education level, and ethnic background. In Loveland, CO, women were more likely than men to report using personal protective measures (Gujral et al. 2007). A survey of 10 counties in Kansas found that while 97% of English-speaking respondents had heard of West Nile virus, only 41% of Spanish-speaking respondents were aware of the disease. Age, education level, and living in an urban vs. rural area were also found to influence likelihood of engaging in personal protective behaviors as well as the source from which WNV information was received (Fox et al. 2006).

Surveys in Ottawa, Canada, indicated a high level (77%) of awareness of West Nile virus among respondents, and 72.5% reported using DEET-based repellants (Wilson et al. 2005). However, people above the age of 51 were less likely to use repellant, although this is the very age group at higher risk of developing severe neuroinvasive disease if infected with WNV. Similarly, over half the respondents who reported being unaware that people over the age of 50 are at greater risk for serious WNV illness in a survey in British Columbia were themselves over 50 years old (Aquino et al. 2004). In contrast, a study in Ithaca, NY, found that respondents over the age of 55 were three times more likely to use at least one WNV preventive measure compared to all other age groups (Tuiten et al. 2009). Follow-up surveys to determine not only overall success of public education but also specific groups or other demographics that require a more tailored or different approach are thus critical for success.

Additional issues with public perception may also compromise implementation of personal protective measures. The EPA conducted an online survey of 3,000 people across the United States to determine their usage of insect repellant and obtain reactions to new labeling that would make it more obvious which insects are repelled and how long the repellant is effective (EPA 2012). While many respondents indicated that their main reasons for using repellant were to avoid discomfort from bites and to protect against diseases (48% and 46%, respectively), the overall level of repellant use was low, with only one-third reporting using repellant most of the time (39%) or some of the time (33%) when outdoors, and 12% using repellant rarely. Although the EPA survey did not ascertain why people avoided using repellants, over one-third of respondents in a British Columbia survey felt that DEET is an environmental hazard, and over a quarter reported their belief that it is unsafe for human use (Aquino et al. 2004). Similar concerns about the safety of DEET were expressed in a survey of Kansas residents (Fox et al. 2006) and in Ithaca, NY (Tuiten et al. 2009). Identifying barriers such as these to changing behaviors in the face of increased education and knowledge about a problem, as well as providing reasonable and effective alternatives, pose a significant challenge to public health and mosquito control programs.

Interagency Cooperation

Federal, state, and county or city agencies can have conflicting goals in terms of wetland management, with mosquito control agencies fearing increased production of mosquitoes from wetlands, and wetland managers concerned with the effects of mosquito control practices on wildlife health and diversity. Wetlands, especially those that dry out seasonally, have often been viewed as little more than mosquito-filled nuisances, but these temporary habitats also support distinctive wildlife assemblages and provide important habitat for rare or threatened species (Collinson et al. 1995; Baber et al. 2004). Site managers are unwilling to expose the naturally-occurring communities in such habitats to the direct and indirect effects of mosquito control activities, especially as a complete list of the species inhabiting a park or natural area is often not known and potential effects of insecticides on all community members cannot be anticipated. For this reason, the *Draft Mosquito and Mosquito-Borne Disease Management Policy* set forth by the U.S. Fish and Wildlife Service National Wildlife Refuge System focuses on treatment in response to health threats from mosquitoes as opposed to nuisance biting, stating, "we will allow populations of native mosquito species to function unimpeded unless they cause a human and/or wildlife health threat" (Federal Register 2007).

The complexity and variability of wetland habitats and their surrounding landscapes requires an interdisciplinary approach to mosquito control that is often lacking, with the unfortunate result that two laudable goals—preserving the ecological integrity and biodiversity of wetlands, and reducing the number of disease-vectoring mosquitoes—can be placed in needless opposition. In some cases, cooperation may require recognizing that the goal of a natural resource agency to maintain biodiversity is not in accord with the goal of a mosquito control agency to remove nuisance-biting mosquitoes, and the public must be educated to that effect. For example, signs may be placed at the entrances of National Wildlife Refuges warning visitors when nuisance-biting mosquito numbers are high (Federal Register 2007). The San Pablo Bay National Wildlife Refuge in California has worked to maintain its wetlands in a natural state, using improved hydrology and vegetation management to reduce mosquito numbers while working with area vector control agencies when needed to achieve low-impact control. In cases where mosquito larval numbers indicate a need for additional treatment, larvicides such as Bs, Bti, and methoprene are used in a targeted fashion, and adulticiding has not been needed in this refuge in the past 10 years (FWS 2011).

7 Additional Mosquito Control Approaches and Tools

Any mosquito larvicides or adulticides used today, regardless of their improved safety for nontarget organisms compared to control agents of the past, will necessarily alter biodiversity and the cycling of nutrients and energy in the aquatic habitats in which they are used. For those who wish to manage a site as a natural area, the problem of how to control mosquitoes—if necessary—while maintaining the natural ecology of their wetland, requires ongoing effort and adaptive management. The approaches and tools below illustrate different techniques and practices that can be used in combination for effective mosquito surveillance and development of a site-specific treatment plan—including deciding whether treatment is warranted at all—that simultaneously achieves the goals of protecting both biodiversity and human well-being.

Site-Specific Knowledge

Once managers move away from a program of treating a given area at regular intervals, it becomes critically important to have detailed knowledge of both the site in question and the mosquito species that inhabit it (Morris 1991). Because mosquito species differ in their life histories, a control method that works on one species may be less effective against another. In a natural wetland producing nuisance mosquitoes, species-specific knowledge of oviposition and larval habitat preferences may allow effective control to be achieved by treating only a small portion of the wetland, as opposed to applying pesticides to the entire area. For example, Mercer et al. (2005) found that abundance of *Aedes*, *Culex*, and *Uranotaenia* mosquito larvae in an Iowa wetland varied with different microhabitat conditions. This allowed the few microhabitats where the majority of mosquitoes developed to be identified and spot-treated, greatly reducing impacts on nontarget organisms as well as the cost and effort involved in pesticide applications. Large numbers of nuisance-biting *Mansonia* or *Coquilletidia* mosquitoes may be controlled better via vegetation management, as these genera do not encounter pesticides applied to the surface due to their habit of obtaining oxygen directly from submerged plant tissues. Public education is important to control peridomestic container-breeding mosquitoes, while mosquitoes inhabiting wastewater treatment ponds can be better controlled by managing vegetation and site hydrology.

Natural Enemies

Predators of Mosquitoes

Mosquitoes are an important part of aquatic and terrestrial food webs; larvae and pupae are eaten by aquatic predators, and the winged adults provide food for upland animals. Mosquitoes are impacted naturally by a wide range of natural enemies, ranging from pathogens such as bacteria, fungi (e.g., *Lagenidium giganteum*), and parasites (e.g., mermithid nematodes) to aquatic invertebrate and vertebrate predators. Many of these natural enemies have potential to be used as biological control agents (reviewed in Mulla et al. 1979; Rodríguez-Castro et al. 2006; Mogi 2007; Quiroz-Martinez & Rodríguez-Castro 2007; Shaalan & Canyon 2009), either through conservation in the habitats where they co-occur with mosquitoes (conservation biological control), or by rearing and releasing native species into habitats with mosquito larvae (augmentation biological control). In the United States, there are many anecdotal reports about the ability of predators such as dragonflies, bats, amphibians, and birds to control mosquitoes, but fewer experimental tests of their true regulatory capacities. Mosquito control using natural enemies has received much attention in countries where there are insufficient resources to support the costs of ongoing chemical or biological pesticide applications.

Most invertebrates that consume mosquito larvae are predators in the orders Hemiptera (true bugs), Coleoptera (beetles), and Odonata (dragonflies and damselflies) that occur naturally in wetland habitats (reviewed in Quiroz-Martinez & Rodríguez-Castro 2007). The juvenile and adult forms of these insects prey on mosquitoes and are top predators in fishless wetlands (Batzer & Wissinger 1996; Culler & Lamp 2009); the highly mobile winged adults also rapidly colonize new habitats where prey is abundant. Phantom midge larvae (*Chaoborus*) have been shown to feed intensively on mosquito larvae; these midges overwinter as larvae in shallow semi-permanent ponds, so they are present and active in spring when mosquito eggs hatch (Helgen 1989). Some aquatic crustacea such as tadpole shrimp (*Triops newberryi*) and cyclopoid copepods as well as predatory flatworms feed effectively on immature mosquitoes (Blaustein 1990; Tietze et al. 1994; Su & Mulla 2002). Copepods are especially good predators as they appear in a habitat soon after flooding (Mulla et al. 1984a), and they attack and kill a greater number of mosquito larvae than they actually consume (Kumar & Rao 2003). Adult mosquitoes may comprise a substantial proportion of the diet of different spider species (reviewed in Mogi 2007).

In classical biological control, pest organism populations are reduced by a specialized predator that relies largely if not exclusively on the target taxon for food, and has a high rate of increase relative to the pest. In contrast, the predators described above are generalists, feeding on a variety of prey in addition to mosquitoes; because mosquitoes are not targeted exclusively, the impact of these control agents is potentially diminished. Conversely, the ability of generalist predators to switch to alternative prey when mosquito abundance is low allows them to remain in a habitat even when mosquito numbers fluctuate, and arguments in favor of generalist predators as viable biological control agents have been borne out by a variety of successful case studies (Murdoch et al. 1985).

Compatibility of Natural Enemies with Other Control Agents

Natural enemies that can work in conjunction with applied treatments exert greater and more extended mosquito control while requiring fewer and more infrequent pesticide applications. Mulla et al. (1984a) found that an initial application of Bti or Bs in experimental ponds controlled early instar mosquito larvae, and subsequent treatments were unnecessary due to colonization by a variety of predatory invertebrates that effectively controlled later instar larvae. A diverse assembly of predaceous beetle larvae present at sites treated with Bti not only extended control of an initial Bti treatment, but also caused a decrease in mosquito larval numbers prior to Bti application (Mulligan & Schaefer 1981). Bti used in combination with backswimmers (Notonectidae) controlled *Aedes*, *Culex*, and *Anopheles* larvae in artificial pools over a period of 70 days better than Bti alone; addition of backswimmers alone to the containers provided a high level of mosquito control, with a mosquito larval count that would have triggered additional treatments occurring on only a single date (Neri-Barbosa et al. 1997). Salt marsh shrimp (*Palaemonetes varians*) preyed aggressively and preferentially on larvae of *Ae. detritus*, and in microcosms that were also treated with Bti, their fecal pellets were toxic to mosquitoes (Roberts 1995).

Care must be taken to ensure that additional treatments are compatible with the natural enemy in question. For example, the predatory tadpole shrimp *Triops newberryi* was unaffected by high doses of Bti and Bs, but the mosquito larvicide Golden Bear oil caused high mortality (Su & Mulla 2005). A conservation-based mosquito control approach in which native natural enemies are sustained in the habitat can exert effective mosquito control while reducing the number and cost of pesticide treatments required. This strategy also avoids introduction of nonnative predators such as *Gambusia* or repeated pesticide applications, which can reduce populations of native natural enemies, disrupt the wetland food chain, alter the community structure, and even lead to increased mosquito production (Bence 1982, 1988; Culler & Lamp 2009).

Effectiveness of Natural Enemies

The success of natural enemies in mosquito control is affected not only by their direct predation rate, but also by biotic and abiotic factors such as wetland type, hydroperiod, availability of food resources for both mosquitoes and their predators, mosquito larval density, presence of taxa competing for the same resources as mosquitoes, and ability of the control agent to recycle or persist in the environment (reviewed in Kumar & Hwang 2006; Juliano 2007; Quiroz-Martinez & Rodríguez-Castro 2007). Differences among these factors and their inter-relationships explain some of the variation in different studies on

As both adults and nymphs, dragonflies are voracious predators of mosquitoes. (Photograph © Bryan E. Reynolds, www.bryanreynoldsphoto.com.)

biological control of mosquitoes. Bannerjee et al. (2010) suggested that mosquito control efforts be targeted for source site type, using habitat reduction for smaller sites such as artificial containers and drains, which tend to have less overall diversity and thus fewer predators, and alternative control methods in larger more diverse habitats, including ponds and rice fields, where the greater number of predators may already be reducing mosquito populations.

Numerous lab tests and field trials with a range of natural enemies demonstrate their ability to partially or completely control mosquitoes. Dytiscids (predaceous diving beetles), notonectids (backswimmer bugs), and hydrophilids (water scavenger beetles) are among the most voracious invertebrate predators of mosquitoes, and members of these families are common and widely distributed. The highly mobile flying adults are among the first to colonize newly flooded wetlands (McDonald & Buchanan 1981; Mulla et al. 1984a; Walton et al. 1990); the timing of their arrival is critical as they must be present during the window of mosquito larval development before adults have emerged to be effective. Dytiscids often reach high densities in wetlands, and exhibit prey preference and high feeding rates on mosquito larvae (Culler & Lamp 2009), although species with different average body sizes may have different predation rates and preferences for different mosquito larval instars and alternative prey (Lundkvist et al. 2003; Ohba & Takagi 2010). Lab and field tests involving multiple dytiscid species have shown that beetle feeding causes a significant reduction in mosquito larval numbers (McDonald & Buchanan 1981; Lundkvist et al. 2003; Mogi 2007; Chandra et al. 2008; Ohba & Takagi 2010). Backswimmers (Notonectidae) are voracious predators of mosquito larvae, egg rafts, and pupae (Chesson 1984; Saha et al. 2007), and tend to colonize newly flooded sites fairly rapidly, often arriving soon after mosquito larvae are seen (Scott & Murdoch 1983). The ability of different notonectid species to colonize and significantly reduce or eliminate larval and pupal mosquitoes in habitats such as stock troughs and outdoor ponds has been confirmed (McDonald & Buchanan 1981; Chesson 1984; Eitam et al. 2002), and notonectids may also be amenable to mass rearing for release into containers (Rodríguez-Castro et al. 2006).

Both the aquatic nymphs and terrestrial adults of Odonata (dragonflies and damselflies) prey on mosquitoes, but the nymph is the focus of mosquito biocontrol work. Odonates that occur naturally or are introduced into artificial containers have been shown to reduce or eliminate mosquitoes (reviewed in Mogi 2007). Nymphs of five different odonate species (three damselflies and two dragonflies) significantly reduced populations of mosquito larvae in rice paddy processing tanks, even though each species differed in its mosquito predation rate (Mandal et al. 2008). Locally collected nymphs of the hairy dragonfly (*Brachytron pratense*) dramatically decreased densities of the malaria vector *An. subpictus* in outdoor rice paddy processing tanks in India (Chatterjee et al. 2007), and nymphs of native dragonflies and damselflies that occur naturally in tree holes in Panama suppressed mosquito populations in pots and artificial tree holes (Fincke et al. 1997). Monthly introduction of nymphs of a native dragonfly (*Crocothemis servilia*) into domestic water containers in a village in the Yangon region of Myanmar (Burma) during monsoon season reduced *Ae. aegypti* so effectively that many participants requested continued release of nymphs after the study ended (Sebastian et al. 1990).

Although frequently overlooked due to their small size, the freshwater crustaceans known as cyclopoid copepods have enormous mosquito control capacity in some settings and have been used successfully as control agents. Copepods are tiny (~1–2 mm; 0.04–0.08 in.) but aggressive predators, with the added advantages of occurring naturally at high abundance in many wetlands, undergoing diapause when food abundance is low or the habitat dries, and being easy and inexpensive to culture and transport in large numbers (reviewed in Marten & Reid 2007). The larger species are most effective against *Aedes larvae*, though less able to kill and control *Anopheles* or *Culex* mosquitoes. They are sensitive to pesticides such as temephos and pyrethroids, but can be used conjunction with Bti and larvicidal oils (Marten et al. 1993). In a study in New Orleans, a combination of Bti and copepods not only controlled mosquito larvae within a few days but also prevented any recurrence, whereas mosquito populations resurged within ten days after treatment with Bti alone (Marten et al. 1993). Successful mosquito control using copepods has been achieved for container-breeding mosquitoes as well as for those in rice fields, small marshes, and roadside ditches (Marten et al. 1993, 2000; Marten, Bordes et al. 1994; Marten, Borjas et al. 1994; Kay & Nam 2005; reviewed in Marten & Reid 2007), and they have been used by the New Orleans Mosquito Control Board to eliminate mosquitoes in old tires (Marten et al. 1997).

The successful use of copepods as a mosquito control agent by the New Orleans Mosquito Control Board prompted the New Jersey State Mosquito Control Commission to explore their efficacy against the container-breeding *Cx. pipens* that is the major vector of WNV in that state. They successfully colonized a native *Macrocyclops* species known to be an effective mosquito control agent, and found that the copepods controlled mosquito larvae in old tires and were able to overwinter successfully. Continuing field studies are currently underway with participating county mosquito control agencies to examine the efficacy of copepods in additional habitats such as artificial containers and ponds, especially orna-

Studies show that salamanders can significantly reduce mosquito populations, even in temporary pools, which these mobile predators can quickly colonize. (Photograph: David Cappaert, Michigan State University, Bugwood.org.)

mental ponds at homes that were abandoned during the recent economic downturn (Robert Kent, Office of Mosquito Control Coordination, Trenton, NJ, pers. comm., October 2012).

Belostomatidae (giant water bugs) also have potential for mosquito control. In laboratory studies, the giant water bug, *Sphaerodema annulatum*, consumed an average of almost 90 mosquito larvae (*Cx. quinquefasciatus*) per day across a seven-day trial, causing a significant decrease in mosquito pupation rate and adult emergence (Aditya et al. 2004). Similar studies using belostomatids in the genus *Diplonychus* revealed a high predation rate, with individual bugs consuming up to 122 fourth-instar *Culex* larvae per day (Saha et al. 2007).

Naturally occurring vertebrate predators of mosquitoes can limit or control mosquito production. Salamanders are generalist predators that can reach very high densities, and Diptera may comprise up to 60% of their diet (Taylor et al. 1988). A four-year study of twenty-four Indiana wetlands found a 10-fold reduction in mosquito larvae at wetlands inhabited by salamander larvae compared to wetlands without salamanders (Brodman et al. 2003), and larval spotted salamanders (*Ambystoma maculatum*) reduced mosquito survival in mesocosms and seasonal pools (Rubbo et al. 2011). Some salamanders exhibited prey choice in laboratory studies, with microcrustaceans and mosquito larvae and pupae as their preferred foods (Brodman et al. 2003; Brodman & Dorton 2006). In laboratory feeding trials, adult red-spotted newts (*Notophthalmus viridescens*) and larval mole salamanders (*Ambystoma talpoideum*) consistently ate large numbers of mosquito larvae (DuRant & Hopkins 2008), suggesting a significant role in mosquito control in ephemeral wetlands, especially as they can quickly colonize newly flooded habitats (Gibbons et al. 2006). Tadpoles of four native Australian frog species did not control *Cx. annulirostris* populations in laboratory tests, but the combination of predation, competition for resources, and potential oviposition deterrence could contribute to reduced growth and survival of mosquito larvae in the field (Willems et al. 2005). Red-eared slider turtles (*Trachemys scripta*) can prey heavily on late-instar mosquito larvae (Borjas et al. 1993), and have been used to control mosquitoes in water storage tanks in the Honduras and in roadside ditches in Louisiana (reviewed in Marten 2007).

Native fish such as desert pupfish (*Cyprinodon macularius*) and sticklebacks (*Gasterosteus aculeatus*) have the potential to control mosquitoes in the habitats where they occur (reviewed in Garcia 1983). Restoration of natural hydrology to Atlantic coastal salt marshes via Open Marsh Water Management has restored native fish populations that successfully control mosquitoes (see "Restoring Natural Enemies to Salt Marshes" below). Small fish have been used to eliminate *Ae. aegypti* from domestic water containers (Neng et al. 1987). Native fish may be used as control agents in artificial wetlands; a single introduction of native minnows into stormwater ditches successfully suppressed *Culex* mosquitoes (Irwin & Paskewitz 2009), providing an effective and cheaper alternative to the regular VectoLex (Bs) applications normally used. In habitats where *Gambusia* cannot be released due to environmental concerns, other species have been used successfully instead. In New Jersey, native species such as fathead minnow (*Pimephales promelas*), freshwater killfish (*Fundulus diaphanus*), pumpkinseed sunfish (*Lepomis gibbosus*), and bluegill sunfish (*Lepomis macrochirus*) are raised at hatcheries and provided free of charge to county mosquito control districts who follow the New Jersey Department of Environmental Protection BMPs for mosquito control, for use in sites shown to be chronic producers of mosquitoes (Robert Kent, Office of Mosquito Control Coordination, Trenton, NJ, pers. comm., October 2012).

Healthy Wetlands Sustain Natural Enemies

Wetland communities are dynamic, changing as flooding and drying exert different levels of stress; the hydroperiod of a site helps structure the invertebrate community that inhabits it. Some taxa may colonize a newly flooded habitat rapidly, while others find the same site attractive only after a prey base has formed. Resistant taxa are adapted to handle desiccation, persisting in a seasonal wetland in a drought-resistant life stage or maturing and leaving the site for more suitable habitat nearby, while other taxa will die if the site dries down. The longer hydroperiod of permanent wetlands can sustain more diverse predator populations that may exert partial or complete mosquito control. Some researchers have found that mosquito abundance was limited at permanent wetlands due to continuous predation by established populations of natural enemies (Chase and Knight 2003), and others have suggested that mosquito control in a region could be improved by creating permanent open wetlands in the landscape that favor a diversity of predaceous diving beetles (Schäfer et al. 2006), which have good colonization abilities. Breitfuss (2005) found 100 times greater production of *Cx. annulirostris* in temporary rain-filled pools compared to permanent habitats, and other studies have indicated that ephemeral pools are associated with the highest risks of mosquito-borne disease transmission (reviewed in Dale & Knight 2008).

However, temporary wetlands can also sustain a diverse community of predators that may control mosquitoes. Predator taxa were abundant and widespread in ephemeral pools in Western Australia, and a relationship was seen between reduced mosquito density and increased predator richness (Carver et al. 2010), though predator colonization rate after rainfall lagged behind that of mosquitoes at the same sites. Similarly, Walton et al. (1990) found that populations of *Culex* larvae declined sharply 2–3 weeks after habitat flooding, due to the sequential appearance of tadpole shrimp (*Triops*), beetle larvae, dragonfly and damselfly nymphs, and backswimmers. Their results suggest that use of mosquito larvicides should be limited to the period immediately following habitat flooding to control mosquito abundance during the lag period before predators have colonized and attained useful numbers. Chase and Knight (2003) found that mosquito production in temporary wetlands could be limited by co-occurring organisms that are adapted to regular drying and compete with larvae for resources. They further considered semi-permanent wetlands that dry down in drought years to have the greatest potential for mosquito population outbreaks, as the drying process eliminates or disperses all the previously occurring natural enemies, and rapid colonization by mosquitoes upon rewetting allows them to grow unchecked for a time. This process may be exacerbated by the fact that female mosquitoes often preferentially select predator-free habitats in which to lay their eggs (see "Indirect Effects of Predators" below).

Temporary or seasonal wetlands, such as these vernal pools in California, are often home to unusual invertebrate species. (Photograph: Matthew Shepherd/The Xerces Society.)

Temporary wetlands serve as sources of colonists that immigrate to, and breed in, other mosquito-producing sites in the area. However, with increasing wetland destruction and alteration, many wetlands are more isolated in the landscape than they were historically. This habitat isolation impacts community structure and has a negative effect on predator richness (Shulman & Chase 2007; Chase & Shulman 2009), as many predators require larger or more connected areas to maintain healthy populations. A survey of natural ponds with differing degrees of isolation from neighboring wetlands in the region showed a decrease in amphibian and invertebrate predator biomass and an increase in mosquito biomass with increasing isolation (Chase & Shulman 2009). Similarly, recruitment and species richness of dragonflies in cattle tanks was negatively correlated with increasing isolation from source wetlands (McCauley 2006).

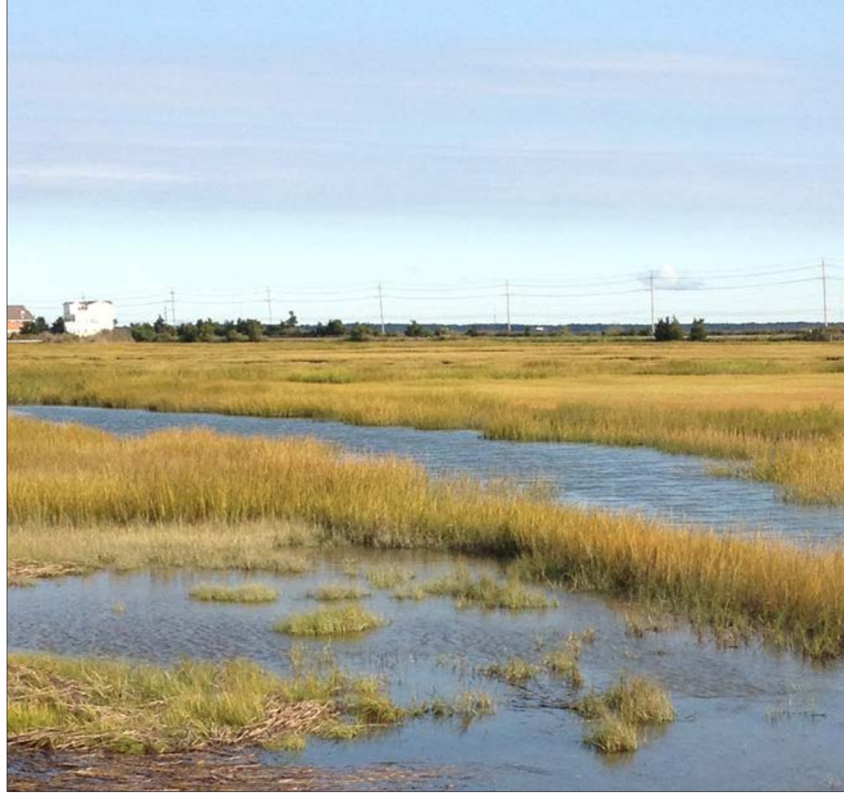
Temporary wetlands can harbor rare or endemic aquatic species. Assemblages of amphibians and aquatic invertebrates in a variety of freshwater wetlands in New Hampshire had greater diversity with increased length of wetland hydroperiod, but some taxa that were found much more commonly in the relatively species-poor temporary wetlands were absent from sites that had a longer hydroperiod and greater overall species diversity (Baber et al. 2004). Preserving the natural community in temporary wetlands to the greatest extent possible is thus desirable in terms of both general conservation, as they may harbor rare or threatened species such as fairy shrimp, as a well as for continuing mosquito control.

Restoring Natural Enemies to Salt Marshes

Specific types of habitat modification are done in previously altered salt marshes and estuaries to restore natural enemies to the system. Salt marshes have historically been a problem for mosquito control as their large area often precludes effective chemical treatments, and they can produce an abundance of mosquitoes that disperse many miles from the source. Early control efforts focused on ditching and draining, but this often resulted in even greater problems as it created many small scattered pools of standing water that were difficult to find and treat, in addition to causing major disruptions in marsh hydrology and ecology (Patterson 2009). Coastal wetlands that were impounded to prevent seawater from entering sometimes created new breeding sites for freshwater mosquitoes (Slaff & Crans 1982).

Open Marsh Water Management (OMWM) is a form of habitat modification designed to restore salt marsh hydrology while simultaneously facilitating natural control of mosquitoes. OMWM reverses the effects of earlier ditching and restores natural daily tidal flow to the wetland, reconnecting isolated pools to tidal inlets. This helps reduce mosquito populations in multiple ways: tidal flows enable access for native fish that prey on mosquitoes, water flow as the tide recedes flushes out mosquito larvae, and the larger deeper pools sustained by regular tidal flow retain fish and are more likely to be colonized by additional invertebrate predators (Dale & Hulsman 1990; Carlson et al. 1999; Meredith & Lesser 2007; James-Pirri et al. 2012). A related technique is Rotational Impoundment Management (RIM), in which water levels are controlled such that the marsh is minimally flooded during the summer, and then reconnected to the estuary (Carlson & O'Bryan 1988).

Multiple studies have shown that OMWM reduces or eliminates pest mosquitoes, in many cases allowing pesticide use to be discontinued (Ferrigno & Jobbins 1968; Telford & Rucker 1973; Resh & Balling 1983; Hruby et al. 1985). In Delaware, OMWM techniques applied in tidal wetlands with the greatest mosquito production have resulted consistently in >90% reduction in larval mosquito populations, dramatically reducing the need for larviciding at sites that previously received intensive annual spraying (Meredith & Lessing 2007). In the early 1980s, Essex County, MA, adapted OMWM techniques used successfully in surrounding states to restore habitat for wading birds and reduce mosquito problems, with a focus on areas of heavy mosquito production; fish inhabiting the new OMWM system subsequently provided 80–100% of all mosquito larval control needed (Jack Card, NE Massachusetts Mosquito & Wetlands Management District, pers. comm., November 3, 2011). In addition to providing effective mosquito control, OMWM has the added benefits of restoring foraging and resting habitat for waterbirds (Clarke et al. 1984) and providing nursery areas for fish, crab, and shrimp, although a shift from fish-dominated to shrimp-dominated communities was seen at a few sites in a recent study of Atlantic coast wildlife refuges, which could result locally in reduced mosquito control (James-Pirri et al. 2012).



A century ago, salt marshes were being ditched and drained. Now mosquito management focuses on restoring daily tidal flows. (Photograph: Celeste Mazzacano/The Xerces Society.)

Indirect Effects of Predators

Predators that reduce mosquito numbers by direct consumption have additional indirect impacts on mosquito populations. The increased risk to mosquito larvae when predators are present in the habitat may lead them to adopt defensive behaviors such as decreased foraging for food, increased use of refuges, and decreased movement, which can alter mosquito larval development rate and adult size and reproductive capacity. The stress imposed by predators can intensify the effects of other biotic factors influencing mosquito populations, such as competition for food resources. For example, lab studies showed longer development time and reduction in adult emergence of *Anopheles* mosquitoes reared in the presence of both notonectid predators and trophic competitors (snails and zooplankton; Knight et al. 2004). Nymphs of the dragonfly *Anax imperator* significantly reduced the size of *Cs. longiareolata* at pupation and increased development time for male mosquito larvae (Stav et al. 2005). *Culex pipiens* exhibited decreased survival, slower development, and reduced wing length of emerged adult mosquitoes when reared in water that had been exposed to predatory backswimmers (*Notonecta glauca*) fed on other *Cx. pipiens* (Beketov & Liess, 2007); these effects were significantly stronger for female mosquitoes. This suggests that predators fed conspecific mosquito larvae generate a chemical cue that induces behavior changes in mosquitoes, even in the absence of the predator itself, especially as the effects were less pronounced when mosquito larvae were reared in water that was exposed to *Notonecta* fed on an alternate, non-mosquito prey (*Daphnia*).

Chemicals produced by predators may be sensed by gravid females and deter oviposition into the habitat. Female *Culiseta* and *Culex* mosquitoes significantly reduce oviposition into habitats where predators such as backswimmers (*Notonecta*, *Anisops*), amphibians (tadpoles, salamanders), or fish (*Gambusia*) are present (Chesson 1984; Blaustein & Kotler 1993; Blaustein 1998; Angelon & Patrenka 2002; Eitam et al. 2002; Kiflawi et al. 2003; Eitam & Blaustein 2004; Rubbo et al. 2011), and *Cs. longiareolata* females avoided laying eggs in pools with nymphs of *Anax imperator* dragonflies (Stav et al. 1999, 2000). Interestingly, a study of the predatory copepod *Mesocyclops longisetus*, which is known to colonize small containers, suggested that chemicals released by the copepod acted as oviposition attractants for *Ae. aegypti* females (Torres-Estrada et al. 2001), which could be advantageous for biological control.

GIS Surveillance

Accurate surveillance and sampling in an appropriate geographic setting is a critical component of rational mosquito management (Nelson 1994), but traditional on-the-ground assessment of larval habitats and mosquito populations requires a great deal of staff time and resources, and may not be feasible over large areas (Washino & Wood 1994). GIS (Geographic Information System) is an important tool in targeted mosquito control, as it enables accurate, cost-effective, and rapid assessment of potential mosquito larval habitats that is vital for targeting areas for mosquito sampling and control and assessing potential disease risks. GIS surveillance provides a more comprehensive system of mapping than is possible on the ground with limited staff and resources (Dale et al. 1998), and can enable identification and targeted treatment of only those hotspots where mosquito production is a true problem. Such targeted treatment is cost-effective because less pesticide is needed over the season, and better for biodiversity, as the initial impacts on nontarget organisms will be reduced compared to treatment of the entire site and time for recolonization of the site by affected taxa is decreased since they can persist in nearby untreated zones.

Factors that strongly affect mosquito development such as water, vegetation, and surrounding land use can be identified from available remotely sensed data and used to develop locally or regionally targeted control plans for different mosquito species. Remote sensing in mosquito control has received much attention in malaria epidemiology (Washino & Wood 1994; Hay et al. 1998; Foley et al. 2010), but is widely applicable for nuisance and vector mosquito control in general. Many vector control agencies use GIS to map sites where spraying is done, but it is less common to use it as a proactive, predictive tool to help pinpoint habitat areas for more timely, species-specific control to determine when treatment is necessary, and to correlate centers of human population with mosquito production sites. While local or regional mosquito control agencies may lack the necessary GIS expertise or image analysis facilities, collaboration with local government offices can provide access to staff with GIS skills as well as data from digitized aerial photography, radar sensing, or thermal mapping.

One of the earliest examples of GIS technology in mosquito control used color infrared (CIR) aerial photography to identify vegetation types known to dominate the preferred habitat of saltmarsh mosquitoes (NASA 1973). Similar use of CIR aerial photography in a Michigan mosquito control district facilitated identification of breeding habitats for *Aedes* and *Culex* mosquitoes, which was combined with information on human population centers to develop a targeted treatment scheme that controlled mosquitoes effectively while reducing the total area treated and avoiding broadcast spraying (Wagner et al. 1979). Ozdenerol et al. (2008) used a combination of data from mosquito traps placed by a vector control agency and available GIS environmental data such as elevation, slope, vegetation, land use, land cover, temperature, precipitation, soils, and forest distribution to build a descriptive model of the most likely habitat for *Cx. pipiens* and *Cx. quinquefasciatus* in Shelby County, TN. By combining environmental data with data from mosquito surveillance and human populations, they were able to pinpoint highly suitable habitat for these WNV vector species. As climatic variables changed during the summer, so too did the location of this ideal habitat, although some core areas remained highly suitable across the entire season. Monitoring changes over time in the most likely mosquito habitat is an important tool for in creating a targeted and responsive IPM plan, and in focusing education and outreach in community-based mosquito control on the appropriate audiences.

GIS mapping was done to identify potential habitat for *Cx. tarsalis*, a WNV vector species, in a region of Wyoming where coalbed methane extracted via a "dewatering" process created multiple new impoundments that could become mosquito breeding grounds (Zou et al. 2006). GIS layers showing characteristics of water, vegetation, soil, and topography across a large area were overlaid to pinpoint the most likely mosquito breeding sites. Field validation of the model's predictions showed better than 70% accuracy for water bodies over 0.8 hectares, and this system could be improved by aerial imagery with better resolution. It is important to note that one reason this approach was successful was because the researchers had detailed knowledge of the habitat preferences of the target mosquito. *Culex tarsalis* breeds in small areas of standing water (usually less than 4 ha) that have vegetated edges, are high in organic matter, and lack wave action or flow (Laird 1988; Reisen 1993). Data from remote sensing in Brisbane, Australia, were used to identify potential breeding sites for *Cx. annulirostris*, a vector species whose ability to breed in a variety of habitats makes it difficult to control. Ground-truthing visits showed that 75% of the sites identified as potential breeding sites were correctly identified, even though the aerial photos available had been taken during the dry season. Moreover, although *Cx. annulirostris* were found most frequently in natural wetlands (permanent or temporary ponds in grassy fields or marshes), almost half of the natural wetlands identified in the study lacked mosquito larvae entirely, while all of the artificial or constructed wet habitats (tire tracks, containers, drains) contained mosquito larvae of some species (Dale & Morris 1996). In such a situation, broadcast treatment of all wet sites is neither necessary nor cost-effective.

Vegetation Management in Constructed or Highly Managed Wetlands

Wetlands choked with emergent and floating vegetation can become fertile mosquito breeding grounds, as mosquito larvae find refuge in dense vegetation from predators and wind action (waves make it difficult for female mosquitoes to lay eggs, and can interfere with the ability of larvae to breathe at the water's surface). This issue has been recognized among stormwater professionals, and investigations of stormwater management structures that differ in their mosquito producing abilities show that stormwater ponds with shallow sides, uniform and shallow water depths (i.e., less than 6 in. [15 cm] deep), little or no flow, few predators, and thick vegetation are much more likely to produce large numbers of mosquitoes (Walton 2003; Bradley & Kuntz 2006). Mosquito production may also differ depending on the dominant types of plants at a site (Orr & Resh 1991; Jiannino & Walton 2004), and some wetland plant species have even been ranked according to their contribution to mosquito production (Collins & Resh 1989). Water treatment and detention wetlands that are steep-sided, have less than 20% of the basin covered by vegetation, and provide for different levels of water and flow rates, including deeper pools where a diverse community of vertebrate and invertebrate predators can establish, are critical for decreased mosquito production and can create sites where additional mosquito control is rarely needed (reviewed in Knight et al. 2003; Walton 2003).

Vegetation management regimens in stormwater treatment sites can be optimized to reduce mosquito production. A technique used in stormwater treatment wetlands involves draining the site, knocking down the emergent vegetation and allowing it to dry for a short time, then reflooding the site. This is thought to increase the numbers of denitrifying bacteria, but the resulting organic enrichment after reinundation can cause high mosquito production (Walton & Jiannino 2005). Studies done by Sanford et al. (2003) showed that this technique can be modified to significantly reduce mosquito abundance by allowing the harvested vegetation to dry for a longer period prior to reflooding (i.e., five weeks instead of two). Thullen et al. (2002) found that reducing vegetated areas to hummocks surrounded by open water decreased larval mosquito habitat while increasing habitat for predators, resulting in a 100-fold decrease in adult mosquito emergence while still providing enough vegetation for water treatment purposes (i.e., ammonia-nitrogen removal). Walton (2003) recommended incorporation of deep-water, plant-free zones in constructed wetlands as a more effective mosquito control than either maintaining wetlands with uniform shallow water

Stormwater retention ponds and other artifical wetlands are often close to where people live or work. Vegetation management in these wetlands is a central concern. (Photograph: Matthew Shepherd/The Xerces Society.)

levels and dense vegetation, or routine drying and harvesting of wetland plants.

Wetland vegetation management is often done in constructed or highly managed natural wetlands to improve habitat for waterfowl. Several studies suggest that, with knowledge of local mosquito taxa and their habitat preferences and life histories, vegetation management can be tailored to sustain waterfowl while reducing mosquito numbers and helping to increase abundance of other macroinvertebrates eaten by waterfowl (Batzer & Resh 1992a, 1992b; de Szalay et al. 1995). One recommendation is to use a hemi-marsh configuration, in which half of the marsh is covered by vegetation, often in islands, and half is covered by deep water zones (Walton 2003). Batzer & Resh (1992b) found that mowing heavily vegetated areas of seasonal wetlands created more open water habitat for birds and forced larvae of *Ae. melanimon* and *Cs. inornata* to concentrate along strips of vegetation left at the edges, where treatment efforts could be targeted. Abundances of a predatory dytiscid beetle (*Agabus*) and non-biting midge (*Chironomus*), both of which are eaten by ducks, were also higher in mowed wetlands (Batzer & Resh 1992b). Reduced abundance of *Anopheles* mosquitoes was achieved in a California wetland via targeted harvesting of dense beds of parrotfeather (*Myriophyllum aquaticum*), a preferred substrate for mosquito oviposition and good larval habitat (Orr & Resh 1991). This study highlighted the patchiness of mosquito-generating microhabitats within a site and the efficacy of identifying and treating hotspots as opposed to whole-site treatment. Similarly, reduction of joint grass (*Paspalum distichum*) cover in California seasonal wetlands by anywhere from 20–70% greatly reduced mosquito larvae and pupae produced (by 85% and 95% respectively) across six different species of *Culiseta* and *Culex* mosquitoes (Lawler et al. 2007). In the case of *Cq. perturbans*, a species found more frequently in perennial wetlands with stagnant water, temporary drawdowns done to help stimulate aerobic decay of accumulated organic material also reduced mosquito numbers for several years afterwards (Batzer & Resh 1992b). This was likely due to the fact that "water roots" of cattail plants (into which these larvae insert their respiratory siphons to obtain oxygen) drop off in the presence of higher oxygen and it may take several years for oxygen levels to fall to the point where water roots are produced and *Coquillettidia* recolonize.

Existing vegetation management methods to benefit waterfowl may be adapted to serve the additional purpose of mosquito control. A study of the effects of mosquito control pesticides on a leaf-cutting beetle, *Galerucella calmariensis*, used as a biocontrol agent against invasive purple loosestrife (*Lythrum salicaria*), noted that the deeper impoundments created to slow loosestrife seedling development and support native plant growth for waterfowl also sustained fish populations that effectively controlled mosquitoes (Lowe & Hershberger 2004). An examination of common vegetation control methods used for waterfowl management found that discing or burning reduced the abundance of mosquito larvae in three species in different genera (*Cx. tarsalis*, *Cu. inornata*, and *Ae. melanimon*; de Szalay et al. 1995). Because birds and the nontarget invertebrates they feed on thrive best in habitat with both open water and vegetation, the authors recommended a mosaic approach in which areas of vegetated habitat producing the most mosquitoes are targeted for treatment. Colonization of newly flooded seasonal wetlands can also be affected by the amount of plant cover (de Szalay & Resh 2000), with greater plant cover correlating with increased mosquito colonization but decreased colonization by non-biting midges (Chironomidae), water boatmen (Corixidae) and water scavenger beetles (Hydrophilidae). These results illustrate the potentially widespread impacts of vegetation management, as all three of the latter groups provide a food resource for waterfowl, and water scavenger beetles are effective predators of mosquito larvae.

Bait Traps

Insecticides used to control adult mosquitoes are more broad spectrum than those for larval control, and impacts on nontarget organisms are correspondingly greater. Traps baited with semiochemicals—signaling molecules such as pheromones, feeding stimulants, aggregation pheromones, and oviposition attractants—have been used with success for years to control adults of some crop pest insects, and some researchers have attempted to adapt this methodology for mosquito control. Female mosquitoes are attracted by substances in the exhaled breath of potential bloodmeal sources, especially carbon dioxide (CO_2) and octenol (1-octen-3-ol) (Takken & Kline 1989). Light- and CO_2-based traps such as the New Jersey Light Trap (Mulhern 1942), Centers for Disease Control miniature light trap (Sudia & Chamberlain 1962), and subsequent modifications have been used for decades in mosquito surveillance to obtain population data used to make pesticide application decisions. This technique has not been exploited further for mosquito control, in part because mass trapping is considered likely to be ineffective against organisms like mosquitoes that achieve high population densities (Kline 2006, Kline 2007). However, interest in using attractant-based traps and targets for "attracticide" (lure and kill) mosquito control was stimulated in the United States following the successful use of insecticide-impregnated traps to con-

A variety of traps are available for backyard use, most of which are baited with host-odor attractants, like the one shown here. Despite their apparent success, their use does not always result in less biting.(Photograph © istockphoto/ritajaco.)

trol tsetse fly in Africa (Vale et al. 1993). Mosquito traps include products such as Dragonfly® (BioSesensory, Inc.), Mosquito Magnet® (American Biophysics), Mega-Catch® (EnviroSafe Technologies), and SkeeterVac® (Blue Rhino). The traps are baited with CO_2, octenol and other host-odor attractants, and/or heat, and powered by propane burners, electricity, or batteries.

The efficacy of attractant-baited targets impregnated with a pyrethroid pesticide (lambda-cyhalothrin) was assessed for control of the salt marsh mosquito *Ae. taeniorhynchus* on a small barrier island (Key Island) in Florida. The presence of a wildlife preserve on the island precluded the usual pyrethroid spraying for mosquito control, and the demands of a neighboring resort for relief from biting required exploration of alternative controls. In the first phase of the study, mosquito populations were lower in the resort area, which was surrounded by a protective barrier of bait traps, but the difference was not significant (Kline & Lemire 1998). A similar design was used with great success on Marco Island in Florida to protect a residential complex from abundant *Oc. taeniorhynchus* produced in a nearby mangrove swamp, such that a permanent barrier line was installed at the complex at the residents' request (reviewed in Kline 2006). In other tests, Mosquito Magnet® traps with CO_2, heat, and octenol attractants resulted in an 80–90% reduction in the mosquito population on the Atsena Otie Islands in the Gulf of Mexico after three years of continuous use (Kline 2006), and lowered the numbers of the treehole mosquito *Oc. sierrensis* in residential neighborhoods in Salt Lake City, UT (Hougaard & Dickson 1999).

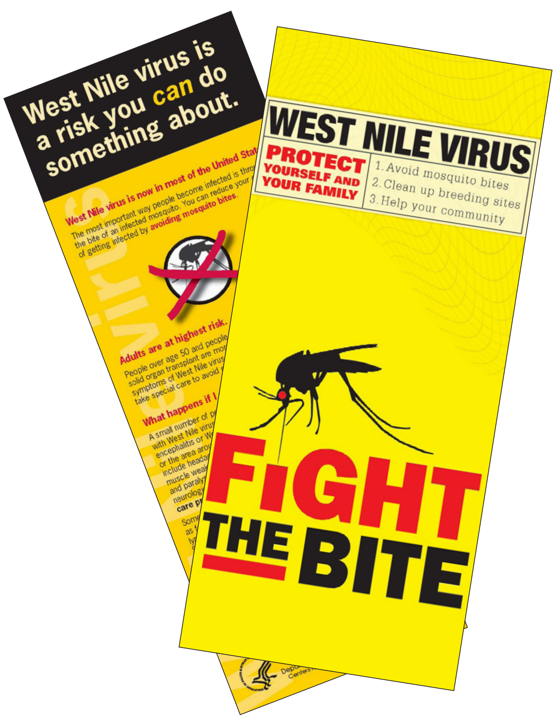
The success of bait traps has been mixed, however. Mass trapping with octenol- and CO_2-supplemented Mosquito Magnet® X traps failed to control *Aedes* and *Culex* mosquitoes in marshes in a Florida state park (Smith et al. 2010), although thousands of mosquitoes were captured. A 14-month field trial of trap and repellant systems in Louisiana showed that the Mosquito Magnet® traps outperformed the Dragonfly/Cognito® system, capturing anywhere from 1.5 to 3.9 times more female mosquitoes at different treatment sites (Collier et al. 2006), although relief from biting pressure in the treatment areas was not measured as a part of this study. However, two repellant systems that were tested concurrently (SC Johnson OFF Mosquito Lantern® and ThermaCell® cordless mosquito system) proved to be highly effective at reducing mosquito numbers in the area; the range of these devices is more limited than that of bait traps (about 21 m^2), but is well-suited to backyard use. There is the possibility that lures may bring in more mosquitoes than the traps can control, and in some cases where high trap counts have been documented, they are not always accompanied by a decrease in mosquito bites. Four Mosquito Magnet® Pro traps placed at rural and urban sites in Manitoba, Canada, collected over two million mosquitoes in six species across ninety-four nights of operation, but landing counts on human subjects did not appear to be significantly reduced (Henderson et al. 2006). Dispersal of large numbers of newly emerged mosquitoes into the test sites may have overwhelmed the traps' catch ability; also, the study measured landing rates on humans at the same time and place where the traps were operating, so the lack of effect may have been due to the fact that traps were continuing to attract mosquitoes into the area.

A relatively nontoxic approach with limited non-target impact is a novel bait which involves mixing boric acid (a stomach poison used against house flies, cockroaches, and ants) into sucrose solutions that are either sprayed onto foliage similar to ULV pesticide sprays or hung in bait traps. In initial laboratory tests, boric acid mixed with a 10% sucrose solution was toxic to mosquitoes with LC_{50}s ranging from 0.1–0.9% boric acid (Xue & Barnard 2003), and sublethal doses reduced survival, host-seeking, and fecundity. Sensitivity differed with sex and species; baits were more toxic overall to male mosquitoes, and less toxic to *An. quadrimaculatus* than to *Ae. albopictus* and *Cx. nigripalpus*. One percent boric acid mixed in a 5% sugar water bait solution and sprayed on vegetation in large outdoor screen cages caused 80–100% mortality among *Ae. albopictus*, *Cx. nigripalpus*, and *Oc. taeniorhynchus* mosquitoes (Xue et al. 2006), and also reduced *Aedes* and *Culex* landing rates on human subjects. This concentration of boric acid was also found to be effective against *Ae. albopictus* in larger outdoor tests (Xue et al. 2011).

These studies suggest that while not a panacea, attracticide traps have the potential to significantly reduce mosquito abundance, especially in areas where a single species dominates the population (allowing optimization of attractants used), or where adult mosquitoes do not disperse far from the larval habitat. Because bait trap use is not widespread, much work remains to be done to optimize trap efficacy for different habitats and species. An important consideration in continuing research is that the number of mosquitoes in an area can change dramatically over the course of a few days regardless of trap use, due to natural cycling of mosquito populations or changes in weather conditions. In addition, if bait such as boric acid is sprayed on foliage instead of hung in traps, persistence and potential effects on nontarget insects must be assessed. While bait traps may not provide complete control, reductions in mosquito abundance can reduce biting pressure on associated human populations in some situations, and decrease the amount and frequency of pesticide applications needed to complete control, especially if used in conjunction with other biorational control methods such as source reduction and personal protection. It should be noted that although optimized for mosquitoes, biting insects such as some midges and biting flies that respond to similar host odors as mosquitoes when seeking a bloodmeal may also be attracted to these traps, but studies are lacking on the extent and effects of trap by-catch.

Public Education

Campaigns to inform and educate the public about mosquito control must be an integral part of any integrated management program. The very real concerns of citizens about mosquito-borne illness are often exacerbated by sensational news reporting and a lack of understanding about the role of different mosquito species in transmitting diseases, the life-history and vector capacity of mosquitoes in a given area, regional breeding areas, infection risk, and options for personal protection and mosquito breeding site reduction. A public education campaign must be developed, delivered, and evaluated using the same scientific principles needed to develop a sound integrated management strategy. Of equal importance is the clear communication to the public of the data and facts in which the control program is based in terms they can understand, as the realization of why a certain action should be taken (or of why nuisance mosquitoes are not being controlled) can enhance support for, and compliance with, an integrated management program.



The Centers for Disease Control has taken a leading role in developing successful public education materials.

In addition, post-treatment surveillance measuring the success of public education programs is just as important as post-treatment surveillance to measure the outcome of a mosquito control treatment. Follow-up surveys must be conducted as part of community education programs, to assess how well the desired messages were disseminated, how effective they were at changing people's behaviors, and to determine ways to improve the materials used in public education and the efficacy of the program. Like mosquito control itself, public education is an ongoing effort; accurate information must be transmitted consistently in a form that is accessible and relevant to the many cultures and socioeconomic levels encountered in a given jurisdiction. The level of outreach effort should be recorded along with changes in human attitudes and behaviors; effects on mosquito populations, biting intensities, and disease transmission levels; and relative cost-effectiveness compared to other control methods used (Nelson 1994).

Public education campaigns have the benefit of enabling a bottom-up system of mosquito control, as citizens are empowered to improve conditions for themselves, their families, and their neighbors. Although gaps between knowledge and practice have been noted, the real efficacy of grassroots vector control efforts has also been demonstrated (see "Public Education Changes Human Behavior and Disease Incidence," page 27).

8 Recommendations for an Optimal Approach to Mosquito Control

An optimal approach to ecologically sound mosquito management requires consideration of several key interconnected elements. There can be no single, scripted mosquito management plan that will have equal efficacy at all sites, but considering the questions below will enable formulation of a site-specific mosquito management plan that balances the needs of the environment with those of the human community.

Educate the Public

An informed public that acts upon their knowledge is critical for mosquito control. Wetland site managers can consult with vector control agencies and state health departments to assess the types of public outreach materials being used to inform the public about the risk of mosquito-borne disease and personal protection measures that can reduce mosquito abundance. This material should also be made available as appropriate at the site if it is visited by the public.

A communication plan for the surrounding community that describes site-specific mosquito management actions and the reasons for their use should be developed. Information should be made available regarding mosquito production at a site as well as whether the species produced are a nuisance or public health threat, or if their dispersal capacity would allow them to move into the surrounding community. Members of the community should be informed that nuisance mosquitoes are not being controlled, what level of biting they may expect at different times of the year, and effective personal protective measures to prevent being bitten at all. Supporting information about the importance to biodiversity of protecting wetland health and sustaining aquatic invertebrate food sources for wildlife while minimizing or eliminating pesticide use will create greater understanding of why "zero tolerance" is not being practiced.

Questions to consider while planning a community edication program include:

Is the site associated with an organization that maintains a web site, Facebook page, Twitter account, or similar? Social media can greatly amplify the extent to which information is disseminated, and provides a venue for individuals to express their comments and concerns.

What different types of electronic and print media are available locally? Using a variety of outlets will ensure that a wider range of people receive the information.

What proportion of the surrounding community does not speak English as a first language and what are the dominant languages spoken? The Centers for Disease Control and state public health departments provide versions of Fight The Bite-type materials in different languages. Translation help may also be obtained from area educational institutions or civic groups.

Are there community-based groups in the surrounding area that can help with disseminating information? In addition, identification of newspapers, radio stations, community groups, businesses, and medical providers that serve different ethnic populations in the area will enable appropriate dissemination of materials, and they may be able to help with translation as well.

Simple signs placed in a community can go a long way toward informing local residents of the steps they can take to protect themselves. (Photograph: iStockphoto/sebastianiov.)

Are there citizen action groups that can help with communication? Such groups may also be able to organize community clean-up operations to remove standing water sources in residential areas.

Are the outreach tools used effective in increasing public knowledge and changing behaviors? An evaluation plan should be developed and implemented to determine the effectiveness of any public education campaign and identify areas of success. The evaluation should include a review of all published materials (both printed and electronic) to ensure suitability of message and presentation, and consideration of demographic groups that require additional outreach or a different approach.

Monitor Consistently and Thoroughly

Monitoring is essential to determine whether a site is producing mosquitoes in significant numbers, to identify the species produced, to assess seasonal patterns of abundance, and to pinpoint microhabitats that are "hotspots" of mosquito production. If biological or chemical insecticides are to be used—for example, in a public health emergency—monitoring is also essential to determine when a defined threshold number of mosquitoes has been exceeded such that treatment of the site is triggered. Finally, monitoring done following site treatment will determine the efficacy of mosquito management and allow for adaptive management as well as validation of the management technique being used.

Monitoring additional animal groups is desirable, for example to determine the suite of natural enemies present at a site and their relationship with seasonal mosquito abundance, and to identify whether sensitive species are present that are likely to be harmed by insecticide use.

Questions to consider when planning a monitoring program include:

Does the site produce mosquitoes? If so, what species are emerging? Note that vector control agencies may have long-term data on mosquito species and patterns of seasonal abundance in the area.

What proportion of mosquito species at the site (if any) bite humans, and what proportion of these are only a nuisance-biting problem as opposed to being disease vectors?

What is the dispersal capacity of mosquitoes from the site? Complaints made by the public to vector control agencies may place the blame for mosquito production on a nearby wetland, when the source of the problem originates in their own back yard.

Does your organization have the authority to decide against treating for nuisance-biting mosquitoes, or can such a decision be over-ridden by vector control agencies? A management practice that involves no treatment of nuisance mosquitoes will be challenged if mosquitoes emerging from the site are moving into nearby residential neighborhoods.

Does the entire site produce mosquitoes at the same rate, or are there hotspots of mosquito production that will be targeted for spot treatment if control is deemed necessary?

What plan for post-treatment monitoring will be implemented to determine efficacy of control measures taken?

What is the composition of the biotic community inhabiting the site? Is there an existing community of mosquito predators such as aquatic beetles, bugs, dragonflies and damselflies, fish, and amphibians?

Does the wetland or its surrounding area sustain any rare, vulnerable, or threatened wildlife species? Does it include animals that depend on aquatic invertebrates such as mosquitoes and midges for food?

Form Cooperative Partnerships

Ecologically sound mosquito control entails a greater knowledge of habitat, wildlife community, mosquito species and life history, and public health, plus ongoing education, monitoring, and surveillance. It is unlikely that any single entity will encompass all of the necessary expertise. Also, site-specific management practices may need to be implemented within a framework of existing local vector control, requiring extensive communication and cooperation with regional vector control agencies. If a mosquito abatement or vector control agency is active in the region, it will be necessary to communicate with them to ascertain the details of their existing mosquito management plan, and to determine the degree of compatibility with practices desired at your site. If site-specific practices are to be implemented, ensure that the vector control agency is made aware of them and their cooperation obtained. An exchange of monitoring and treatment information will also be required, such that the vector control agency may be kept appraised of mosquito abundance and species identity throughout the season.

Irrespective of the site in question, the principles of mosquito management stay the same: monitor thoroughly, know which mosquitoes you are managing, assess the impacts, and prepare a plan—including public education. (Photograph: Celeste Mazzacano/The Xerces Society.)

To identify additional partners needed for optimal mosquito management at a given site, it is useful to ascertain the areas of necessary expertise that are lacking.

Questions to consider when identifying potential partners include:

What is the incidence of mosquito-borne disease in the state and county? These data are available from the Centers for Disease Control and state or county departments of public health. It may be useful to work with, or at least regularly communicate with, a public health department representative in order to be aware of incidence and infection risks for any mosquito-borne diseases in the area, and to receive information regarding the identity of mosquito species implicated as vectors for different diseases.

Does your organization have the entomological expertise to identify mosquito species? Mosquito sampling is a fairly straightforward process, but identification to genus and species requires considerable expertise. An existing vector control agency or public health entity may be able to provide this service as part of their own monitoring program. Additional taxonomic expertise may be available from area universities, environmental consulting organizations, and state or local health agencies.

Are there other nearby wetlands that are also being managed as natural areas? Coordinating planning and actions with federal, state, and county parks staff can help create a more cohesive, consistent management plan across a larger region.

Does your organization have the capacity and technology needed for detailed GIS mapping and surveillance? Government agencies can provide GIS data layers showing important features of potential mosquito habitat (wetlands, stormwater ponds, vegetation, human population centers, disease incidence, etc.), and government agencies or university extension services may aid in creating informative maps for decision-making.

Determine Existing Local Mosquito Control Methods

The existing mosquito control efforts in the area and their compatibility with a desired site-specific management plan must be determined.

Questions to consider while identifying current control efforts include:

Has an integrated management plan been published in the state? If so, how closely does the vector control agency under whose jurisdiction the site falls follow this plan?

What are the most likely environmental impacts of an existing mosquito control plan, and how are they likely to impact the wildlife at the site?

How can you work to get local vector control agencies to allow a site-specific treatment plan that may differ from their own methods, or to adopt different methods of mosquito management, including not treating nuisance-biting mosquitoes?

Create Informative Maps

GIS technology enables detailed data-gathering and the creation of informative maps that can help guide mosquito management. Mapping the data gathered from ongoing monitoring and overlaying it with additional information such as habitat characteristics, rainfall, and temperature enables identification of mosquito-producing hotspots that can be targeted for treatment, portions of habitat that may be amenable to manipulation to reduce mosquito breeding (e.g., vegetation or hydrology management), areas where natural enemies are present, and weather conditions that may lead to increased mosquito production. This information can help prioritize sites (or microhabitats within a single site) annually for adult trapping and larval sampling activities, reveal seasonal patterns, and allow detection of long-term trends or unusual events. (These may include the appearance of a new species within an area or unusually high species abundance compared to multi-year averages.)

Develop and Implement a Site-Specific Management Plan

A plan should be put in place that sets forth the steps to be taken in site treatment, grounded in monitoring data and sound IPM principles.

Issues to be considered while developing an integrated management plan include:

We do not recommend using pesticides for control of nuisance-biting mosquitoes, but we recognize that political or legislative exigencies may override a site-specific decision not to treat nuisance mosquitoes. If a desired outcome of "no treatment" is not possible based on the scope and practice of regional vector control agencies, it will be necessary to work with agency personnel to determine the treatment plan that will have the least impact on the habitat. The agency should provide information on what threshold level of biting pressure will trigger mosquito control, and what data was used to arrive at the threshold number. Increased outreach and education will also be needed if the site is visited or used by the public, to warn them to expect mosquitoes, explain that there is no risk of disease, and encourage them to take personal protective measures.

If treatment measures are deemed necessary, what is the best combination of physical, biological, and chemical controls that can be implemented to achieve effective control while causing the least harm to the wetland?

What species and abundances of mosquitoes will trigger treatment with a biological or chemical pesticide?

Are there management practices that will support conservation biological control by improving site quality and sustaining increased biodiversity, including natural enemies of mosquitoes?

For a site that is producing mosquitoes, are there water or vegetation management practices that could be implemented to reduce mosquito breeding sites? This pertains to artificial or constructed wetlands rather than natural areas, or sites that are already managed for waterfowl habitat or stormwater treatment.

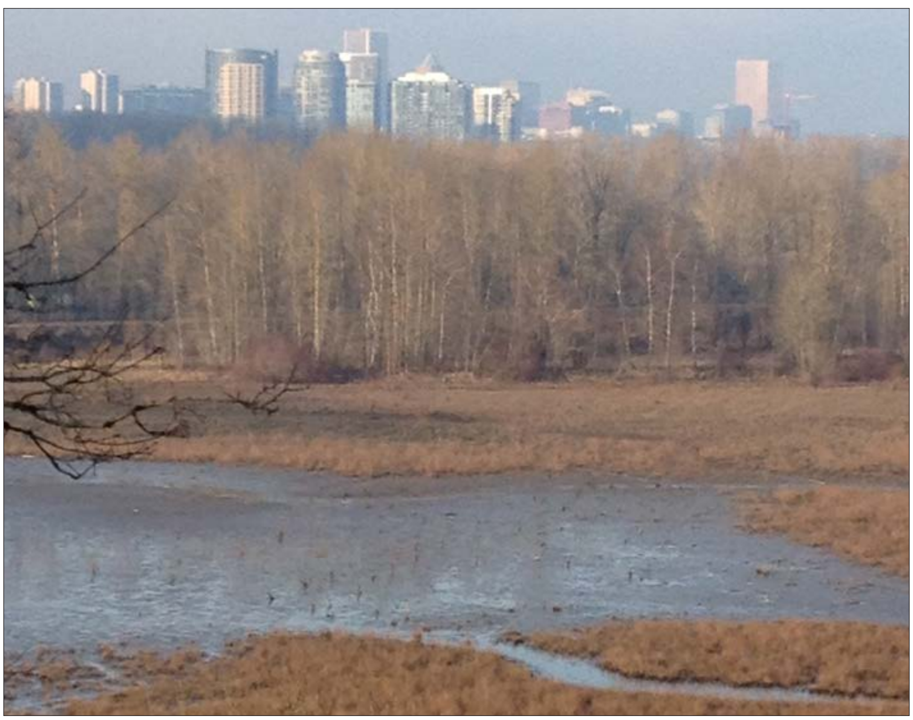
What is the least toxic alternative for treatment, including both type of treatment used and extent of the area to which it is applied? Using least-toxic alternatives applied to those microhabitats where mosquito production is highest will enable control while minimizing the impacts on nontarget organisms and sustaining overall ecosystem health.

9 Conclusions

Mosquitoes are a natural and integral part of wetland ecology, and their extermination is not practical, possible, or necessary. Not all wetlands produce mosquitoes, and those that do may not produce an abundance nor even species that bite humans or vector viruses and other pathogens that cause disease.

The variety of aquatic habitats and mosquito species in North America means that there is no single magic bullet for mosquito control. Experience has shown that broad-scale chemical control harms wildlife and does not provide a long-term solution. Ecologically sound mosquito management will require a mixture of techniques adapted for different geographic regions, habitats, and communities, as well as frequent and effective communication between land managers, mosquito control agencies, and the public.

As with any true IPM program, targeted, ecologically sound mosquito management requires a substantial input of time. Site managers and mosquito control staff need a thorough knowledge of site characteristics, access to entomological expertise, and ability to conduct ongoing surveillance and assessment to determine both treatment needs and efficacy. Effective communication among different agencies is required, along with adaptive management to optimize mosquito control as changes in site characteristics, weather, biotic communities, mosquito populations, and disease incidences may occur. However, such a program has multiple benefits, as it avoids unnecessary treatments, lowers costs by reducing pesticide applications, and is responsive to human health needs, while protecting the incredible diversity of wildlife that are sustained by aquatic habitats.



Mosquitoes are a natural and important part of wetlands, supporting a wide range of other wildlife. No wetland is the same as another, and optimal mosquito management requires a plan tailored to the characteristics of the site. (Photograph: Celeste Mazzacano/The Xerces Society.)

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Appendix A. Mosquito Natural History and Vector Capability

Habitat

Mosquito larvae can be found in fresh, brackish, or salt water; in natural habitats such as ponds, marshes, swamps, lake edges, tree holes, and hoof prints; and in artificial habitats such as cisterns, irrigation ditches, stormwater detention ponds, flower vases, dog bowls, and the puddle under a leaky outdoor faucet. One thing all mosquitoes do have in common is their preference for breeding in standing water.

Development

Floodwater mosquitoes, including species in the genera *Aedes*, *Ochlerotatus*, and *Psorophora*, lay their eggs in damp soil in low-lying areas where they can remain dormant for months until flooded by rainwater or snowmelt, when the subsequent drop in oxygen levels triggers egg hatching. Container-breeding floodwater mosquitoes lay their eggs above the water line in tree holes or artificial containers, where they will be flooded by the rising water after sufficient rainfall (Wallace & Walker 2008). Other mosquitoes such as *Anopheles* and *Culex* species breed in permanent or semi-permanent aquatic habitats, laying their eggs singly (*Anopheles*) or in rafts (*Culex*) on the surface of the water, where they hatch in a few days. *Culex* mosquitoes breed in many different types of standing water, including natural pools and artificial containers, and often prefer water with a high concentration of organic material. *Anopheles* mosquitoes prefer cleaner water and may be found in natural habitats such as wetlands, lakes, and the edges of slow-flowing or drying streams, as well as in artificial containers such as cisterns or irrigation ditches (Gwadz & Collins 1996; Silver 2008; Wallace & Walker 2008).

Eggs hatch into immature forms called larvae, wriggling, threadlike forms that feed on small particles such as organic detritus, microorganisms, algae, and zooplankton. Larvae of *Toxorhynchites* and some species of *Psorophora* are predatory and feed on other mosquito larvae; *Toxorhynchites* has even been used as a biological control agent against pest mosquitoes, with mixed success (reviewed in Garcia 1983; Lacey & Orr 1994; Collins & Blackwell 2000; Shaalan & Canyon 2009). Mosquito larvae develop through four larval stages called instars, molting (shedding their skin) from one instar to the next. At the end of the fourth instar, the mosquito transforms into a non-feeding but mobile pupa, from which the winged adult form emerges. All stages require oxygen, and larvae must return to the surface of the water to breathe; a notable exception is *Mansonia* and *Coquilletidia* mosquitoes, whose larvae and pupae insert their breathing siphon into submerged aquatic plants and obtain oxygen from the plant tissues. Development time is strongly dependent on temperature, and mosquitoes may complete larval development in days to months, depending on environmental conditions, crowding, and food availability (Silver 2008; Wallace & Walker 2008).

As adults, both male and female mosquitoes obtain energy by feeding on plant nectar or rotting fruit. Males do not feed on blood, but the female's eggs cannot mature without the protein provided by a blood meal. Some mosquitoes can produce their first batch of eggs using protein reserves carried over from the immature stage (Spielman 1973), but most are unable to complete egg development without first obtaining a blood meal. A female mosquito generally mates only once (Craig 1967), fertilizing all of her eggs with sperm stored from that mating. She may require multiple blood meals to mature a single batch of eggs or to lay more than one batch, and can lay from 50–200 eggs after feeding. Female mosquitoes feed on a variety of mammals, birds, or amphibians. Different species have different host preferences, and host preference may even vary seasonally. Those that feed on mammals may be zoophilic, preferring to feed on other animals, while others may be anthropophilic, with humans as their preferred host.

Disease Vector Capability

Female mosquitoes of some species may be elevated from nuisance biters to disease vectors based on their bloodsucking behavior. If a female feeds on a host that is infected with a virus or parasite, the organisms may be able to survive or progress through part of their own life cycle inside her body, and can then be injected into the next host as she salivates during feeding. Mosquitoes in the genus *Anopheles* are best known as vectors of the

Plasmodium pathogen that causes malaria, while *Culex* and *Aedes* mosquitoes can transmit viruses that cause encephalitis and dengue. Most species that are zoophilic do not tend to be disease vectors, and often when a human enters the disease cycle they are a dead-end host for the parasite. When hunting for a meal, female mosquitoes home in on volatile chemicals found in the breath and body odors of their prey, including carbon dioxide, and these materials have been exploited as bait in mosquito traps. Because a female may ingest several times her body weight in blood, she often needs to rest and digest for a while after feeding, and pesticide treatment of surfaces near mosquito feeding areas has been used as a means of control.

Abbreviations, Acronyms, and Glossary

Abbreviations and Acronyms

Ae. – *Aedes.*

Ach – Acetylcholine (invertebrate and vertebrate neurotransmitter).

a.i./ac. – Active ingredient per acre.

An. – *Anopheles.*

Bs – *Bacillus sphaericus.*

Bti – *Bacillus thuringiensis* var. *israelensis*.

ChE – Cholinesterase (enzyme in vertebrate and invertebrate nervous systems).

Cx. – *Culex.*

Cq. – *Coquillettidia.*

Cs. – *Culiseta.*

GMO – Genetically modified organism.

ha. – Hectare (1 ha. = 2.47 ac.).

IGR – Insect growth regulator.

IMM – Integrated mosquito management.

IPM – Integrated pest management.

IVM – Integrated vector management.

JH – Juvenile hormone.

kg – Kilogram.

LD_{50} – Lethal dose that kills 50% of test population.

Ma. – *Mansonia.*

Oc. – *Ochlerotatus.*

OC – Organochlorine.

OMWM – Open marsh water management.

OP – Organophosphate.

PBO – Piperonyl butoxide.

ppm – Parts per million.

SIT – Sterile insect technique.

ULV – Ultra-low volume (spray)

Glossary

Abiotic – Nonliving; chemical and physical factors in the environment that affect living organisms (i.e., water, temperature, weather).

Active ingredient (a.i.) – Chemicals in pesticide products that kill, control, or repel the target.

Acute – Single exposure, or short-term exposure.

Adulticide – Pesticides applied specifically against the adult stage of the mosquito; typically applied as ULV spray.

Anthropophilic – In female mosquitoes, a preference to feed on humans.

Biotic – Living factors in the environment.

Chitin – Polysaccharide that forms the structural component of the outer skeleton of insects and crustaceans.

Chronic – Repeated exposures over time.

Cuticle – Outer covering of insects and other invertebrates.

Diapause – Period of time in which growth and development are temporarily suspended; may be seen in conjunction with seasonal changes or development of unfavorable environmental conditions.

Entomopathogenic – Disease agent, such as a fungus, virus, or bacteria that specifically affects insects.

Exoskeleton – External skeleton of insects and other arthropods.

Hydroperiod – General seasonal period of surface inundation; pattern of water level fluctuations in a wetland.

Instar – Developmental stage of an insect or other invertebrate in between each molt; mosquitoes develop through four larval instars before pupating.

Integrated mosquito management (IMM) – IPM program specifically designed for mosquitoes, in which control strategies are used when mosquitoes reach a level at which public health is likely to be compromised.

Integrated pest management (IPM) – Decision-making process that uses environmentally sustainable techniques for pest control. IPM typically combines cultural, physical, biological, and least-toxic chemical control strategies that are applied once regular surveillance determines that pest levels have risen to an economically damaging level.

Integrated vector management (IVM) – IPM program that is used when disease vectors reach a level at which public health is likely to be compromised.

Juvenile hormone – Hormone in insect larvae that controls the rate of development and molting.

Larvicide – Pesticide that acts against the larval stage of a mosquito.

LD_{50} – Dose or concentration of a substance that induces mortality in 50% of the exposed test organisms; the lower the LD_{50}, the more toxic that substance.

Mesocosm – Experimental water enclosure that allows creation of near-natural conditions.

Nontarget organisms – Organisms that are killed unintentionally as the result of pesticide application.

Phytoplankton – Microscopic photosynthetic organisms that inhabit the upper sunlit surfaces of water bodies; primary producers in aquatic ecosystems.

Periphyton – Complex mixture of algae, cyanobacteria, and microbes attached to submerged surfaces in most aquatic ecosystems; important food source for invertebrates, tadpoles, and some fish.

Point source – Any discernible, confined, and discrete conveyance of pollutants to a water body, such as a pipe, ditch, channel, tunnel, conduit, well, container, stormwater conveyance, concentrated animal feeding operation, landfill leachate collection system, or other floating craft from which pollutants are discharged.

Pupacide – Pesticide that acts against the pupal stage of a mosquito.

Sterile insect technique – Insect control based on the release of large number of sterile males, who mate with wild females and thus prevent egg laying. Typically, sterility is induced in the males though exposure to gamma radiation.

Sublethal dose – Dose or concentration of a substance that does not cause significant mortality but may cause other detrimental effects.

Sublethal effect – Physical or behavioral effects on individuals that survive exposure to a pesticide or are exposed to sublethal concentrations.

Terpenoid – Organic chemical derived from 5-carbon isoprene unit; insect juvenile hormone is a terpenoid.

Vector – Carrier, in this context a female mosquito, which transfers an infective agent from one host to another in the course of taking bloodmeals.

Vector control – Any method used to limit or eradicate animals that might transmit disease pathogens.

Zoophilic – In female mosquitoes, a preference to feed on animals other than humans.

Zooplankton – Tiny invertebrates suspended in water bodies; fresh-water zooplankton include microcrustacea (copepods, cladocerans), protozoa, and rotifers.

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