Appendix L. Integrated Pest Management Plan for Mosquito-Associated Threats

Integrated Pest Management Plan for Mosquito-Associated Threats
Stone Lakes National Wildlife Refuge
August, 2006

I. Introduction

Stone Lakes National Wildlife Refuge (Refuge) collaborates with the Sacramento-Yolo Mosquito Vector Control District (District) in monitoring and controlling mosquitoes to ensure the human health concerns of neighboring communities are addressed. The Refuge is located within Sacramento County, 10 miles south of downtown Sacramento and bordered by the city of Elk Grove on the east. The potential for mosquitoes to be produced or harbored on the Refuge is a concern to nearby residents and urbanized areas immediately adjacent to the Refuge are well within the flying range of many species of mosquitoes. Because of this and commitments made by the U.S. Fish and Wildlife Service (Service) in the Final Environmental Impact Statement (1992) establishing the Refuge approved boundary, the Service entered into a Memorandum of Understanding (MOU) in 1993, to ensure the public health and well-being of residents would not be adversely affected by mosquitoes from the Refuge. This Integrated Pest Management (IPM) Plan for Mosquito and Associated Threats will facilitate implementation of the MOU and ensure mosquito management practices are consistent with Service IPM policies and regulations regarding management of the national wildlife refuge (NWR) system.

Refuge Description

Stone Lakes National Wildlife Refuge was established in 1994 becoming the 505th NWR. The approved Refuge boundary encompasses 17,640 acres, of which approximately 6,200 acres are currently managed by the Service. Stone Lakes NWR lies between the Coast and Diablo Ranges to the west and the Sierra Nevada to the east. Most of the Refuge lies within the 100-year floodplain of the Mokelumne and Cosumnes rivers. Interstate highway 5 roughly bisects the Refuge north to south. Annual temperature in the area averages approximately 61.0 F degrees and annual precipitation averages approximately 17.93 inches; virtually all of the precipitation occurs during the winter months. Summer is typically hot (>100 °F) and dry; winter temperatures are generally moderate (50-60 °F). Habitats on the Refuge consist of upland grasslands (55 percent), riparian forest and associated shrublands (7 percent), open water (7%), seasonal and permanent wetlands including vernal pools and irrigated pastures (26 percent), and croplands (5 percent). Land uses adjacent to the Refuge include farming (vineyards, orchards and row crops), grazing, and suburban housing developments.

Memorandum of Understanding (MOU)

The 1993 MOU between the District and the Refuge outlines a mosquito management program that includes consultation on wetland design and water management, use of physical, biological and chemical control agents to control mosquito larvae and adults, and cooperative research on landbird populations. Both the Refuge and the District agree that biological, cultural and physical control methods are preferred over chemical measures and that wetlands can be designed and managed to minimize mosquito production. In summary, the MOU provides for: 1) allowing the District to review planned Refuge wetland construction projects; 2) providing the District an annual summary of the upcoming Refuge water management program and notification of flood ups and irrigations; 3) the District providing a proposed annual mosquito abatement operating plan to the Refuge, 4) the Refuge submitting pesticide use proposals (PUP's), as needed, for any chemical mosquito control agents requested by the District; 5) providing access to the District for mosquito monitoring and control as defined in an annual Special Use Permit (SUP); and 6) with notification and coordination, application of larvicides or adulticides by the District, when treatment thresholds are exceeded.

II. Mosquito Borne Disease

Disease History

Due partly to its climate, California has a history of serious arboviral disease problems that are not expected to diminish. Western equine (WEE) and St. Louis encephalitis (SLE) viruses are endemic and intermittently represent significant public health threats throughout the state. St. Louis encephalitis, historically a rural disease in the western USA, has now moved into the expanding metropolitan areas of southern California. Several international arboviral diseases have recently been introduced to the United States, such as dengue, Venezuelan equine encephalitis, and West Nile virus (WNV). WEE tends to be most serious in very young children, whereas elderly people are most at risk to SLE and WNV (CA Dept. of Heath Services 2003). WEE and WNV can also cause diseases in horses and emus, and WNV kills a wide variety of endemic and imported birds. Mosquito control is practically the only known method of protecting people and animals from WEE, SLE, and WNV. With the exception of available vaccines to protect horses against WEE and WNV, there are no known specific treatments or cures for the diseases caused by these viruses (CA Dept. of Health Services 2004).

West Nile virus was introduced into New York City during 1999 and has subsequently expanded its occurrence dramatically throughout North America. It was first documented as becoming established in California in 2004 when a total of 822 human cases were verified, primarily in southern California (Table 1). Of the 58 counties in the state, 23 reported virus activity during 2004, based on a range of monitoring methods, including: mosquito pools, sentinel chicken flocks, wild birds, or equine and human cases (CA Dept. of Heath Services 2004, CA West Nile Virus website 2006). During 2005, 54 counties were documented as supporting virus activity as it moved northward into the Central Valley and the northern state. In 2005, Sacramento County was recognized as a focus of WNV activity in the state.

Table 1. West Nile virus human cases in California (2004-2006). Numbers in parentheses are totals for Sacramento and Yolo counties.

Year	Human Cases	Human Fatalities
2004	822	25
	(4)	(0)
2005	935	19
	(189)	(1)
2006	50	1
(as of 08/18/06)	(16)	(0)

A number of characteristics of the Sacramento region may have contributed to this, including: (1) number of days per year temperatures exceed 95 degrees Fahrenheit., (2) abundance of mosquito production habitat particularly rice fields, irrigated pasture, dairies, and wetlands, (3) abundance of local migratory bird populations, (4) burgeoning human population interfacing with nearby agricultural lands, (5) heavy spring rains, and (6) the relative abundance of the primary WNV vector, *Culex tarsalis* (P. Sanders, SYMVCD, pers. comm.). During 2004-2005, human cases were documented in the state from approximately the first week of July through the first week of November. To date, there have been 16 human WNV cases in Sacramento and Yolo counties and one mortality in Butte County. WNV activity has been detected in a total of 43 counties. The five major species of birds most commonly found dead and testing positive for WNV in the state have been American crow, Western scrub-jay, yellow-billed magpie, American robin, and house finch.

A statewide encephalitis virus surveillance program has been in place since the 1960s that tracks mosquito abundance and enzootic transmission (transmission within the same geographic area) to provide an early warning of the potential for human infection. WEE and SLE have been recorded in the Central Valley since the 1940s reappearing intermittently after periods of apparent extinction. Though SLE has been rare since 1972, WEE enzootic transmission in the Sacramento Valley appears to be cyclic, recurring at 10 year intervals (Reisen et al. 1995). WEE is transmitted enzootically during the summer between wild birds and *Culex tarsalis*, while *Ocheloratus melanimon* is involved in a secondary cycle with rabbits in the Central Valley during the late summer (Reeves 1990). The mechanisms by which the viruses overwinter and are able to persist despite periods of apparent extinction are still unclear though they have been the subject of intense study for over 50 years (Kramer 1999). There are currently two main hypotheses, one, that WEE overwinters by chronic infection of one or more species of birds, and two, that the virus persists between seasons in adult mosquitoes. Humans and horses are infected tangentially by mosquitoes but are dead-end hosts for the virus. Data collected from county health clinics by the Encephalitis Virus Surveillance indicating a low rate of infection in humans even during periods of elevated enzootic transmission may reflect a lowered rate of mosquito-human contact. This reduction in the exposure of humans to mosquito bites is likely due to cultural factors like the prevalence of televisions and air conditioners that encourage the population to spend more time indoors during dusk, the main period of mosquito activity. Expanded mosquito control and water management programs have also reduced *Culex tarsalis* populations.

Mosquitoes

The mosquito species identified by the District for monitoring and control at the Refuge are *Culex tarsalis*, *Anopheles freeborni, Aedes vexans, Aedes melanimon, Aedes nigromaculis,* and *Aedes increpitus*. *Culex tarsalis* is the primary vector of WEE and SLE in California and is also considered to be a significant vector of WNV (CA Dept. of Health Services 2003). *Anopheles freeborni* can transmit the malaria parasite to humans and is common in the rice growing regions of California. *Aedes melanimon* is involved in the encephalitis virus (sleeping sickness) cycle, and is considered a secondary vector for WNV (SYMVCD 2004).

Mosquito Biology

Mosquitoes are dipterans with aquatic immature stages and an aerial adult stage. Eggs must come in contact with water in order to hatch. Mosquitoes have four aquatic larval stages (instars) plus an aquatic pupal stage. The aerial adult emerges from the pupal stage onto the surface of the water, expands its wings, hardens its exoskeleton, and flies off. It takes from three to 12 days for a mosquito to complete its life cycle in northern California, depending on seasonal and environmental factors and the particular mosquito species involved. In general, the warmer the ambient temperature, the fewer days are required from hatching to emergence.

Ochlerotatus (floodwater) mosquitoes (*O. melanimon, O. nigromaculis, O. vexans*)
The *Aedes* life cycle is initiated with the flooding of ground that has undergone a dry

period. In the Sacramento Valley, the dry period may occur at any time from May through September. Once flooded, eggs that had been laid during the previous wet cycle hatch, pupate, and emerge as adults. Gravid females lay their eggs singly on damp soil, in leaf litter, in cracks in the soil, at the edges of drying ponds, or at the bases of grasses and other plants. Each female lays approximately 150 eggs per ovarian cycle. These eggs are very drought resistant, which allows them to survive during the summer. These mosquito species overwinter in the egg stage and to a much lesser extent in the larval stage. *Aedes* mosquitoes (primarily *A. melanimon*) are the most abundant produced on Refuge habitats, primarily during late summer and fall flooding. Numerous *A. melanimon* can also be produced as a result of wetland irrigations in late spring through early summer. During these times, mosquito eggs hatch, pupate, and reach the adult stage rapidly. *Ochlerotatus* are most numerous on Refuges during the fall when the majority of wetlands are flooded. Multiple hatchings of eggs commonly occur due to the timing of the different broods of eggs and differences in flooding schedules for individual areas. During the warmer months, it generally takes 3-10 days for these mosquitoes to develop from egg to adult (i.e., after initial flooding). Adult females of the three species of *Aedes* mentioned above are all aggressive, relative to other species, and are biters of primarily mammals. During the day, females will bite if disturbed or if a host presents itself, but generally are more active at dusk. Biting and swarming (mating) activities are typically crepuscular (occurring at twilight). When newly emerged, these mosquitoes do not readily move away from their emergence sites. As they age, however, they will move about much more freely. Although all three species of *Aedes* are produced in this area, *A. melanimon* has consistently been the most numerous and the cause of most concern as both as a nuisance and a public health risk. *Aedes* mosquitoes have been documented as secondary vectors for California Encephalitis and WNV.

Culex tarsalis (Encepalitis Mosquito)
Culex tarsalis occur in northern California in very large numbers during the summer. Females lay their eggs on the water surface in bunches called rafts. Each raft contains around 100-150 eggs, hatching about 24 hours after being laid. The immature stages can be found in almost any source of water except treeholes. During the summer, development from egg to adult takes about 9 days in the Sacramento Valley. This species is dramatically multivoltine (producing several broods), with adults emerging continuously throughout the summer. Abundant larva are commonly found in rice fields, poorly drained pastures, wetlands, sewer treatment plants, log decks, dairy farms, and seepages. Within Refuge habitats, *C. tarsalis* can be abundant in seasonal marsh and watergrass production units that have been flooded for more than two weeks during the fall. Adults spend daylight hours resting in secluded places such as cellars or animal burrows. Biting and swarming activities are crepuscular. Peak populations occur in late June or early July. *C. tarsalis* are primarily biters of birds, but will bite humans, livestock, and other mammals if the opportunity presents itself. *C. tarsalis* are strong fliers. Mark-release-recapture studies conducted in Sutter County in 1989 and 1990 showed that adult *C. tarsalis* could move up to 3 miles in just one night. *C. tarsalis* are the primary vector for Western Equine Encephalitis, St. Louis encephalitis, and WNV in humans.

Anopheles freeborni
A. freeborni also occur in northern California and are numerous during the summer. Rice fields are the primary production areas for this species although the immature stages are also found in ditches, seepages, sloughs, and wetlands. Females lay their eggs singly on the surface of the water where they hatch approximately 24 hours later. On the average, it takes 12 days for *A. freeborni* to develop from egg to adult in the Sacramento Valley. *A. freeborni* are most abundant in persistent wetlands, thus Refuge perennial marsh can produce *A. freeborni* during the summer months. However, relatively stable water levels and a relative abundance of mosquito predators (fish, dragonflies, and aquatic beetles) tend to minimize

adult mosquito emergence from these habitats.

This species is also multivoltine, with the ability to produce a continuous supply of newly emerged adults under the right habitat conditions. Adults rest during the day and engage in biting and swarming activities during crepuscular periods. In the fall, females go into diapause (overwintering stage) until January, February, or March when they come out of diapause and seek blood meals on warm days. After obtaining a blood meal, many females resume their overwintering stage until April or May. *A. freeborni* populations peak in late July or August. The females will readily bite humans and livestock. Area-wide, they are the most common nuisance mosquito for humans. Studies on *A. freeborni* in California have indicated long flight ranges from source areas. Work done in Sutter County found that this species could fly 2-3 miles in one night. This species is considered to be the most important vector of malaria in the Western United States.

III. Monitoring Mosquito Populations

District monitoring activities are designed to assess the abundance of immature (larvae and pupae) and adult mosquito populations. Monitoring activities conducted on the Refuge may include: larval sampling, adult light and host-seeking traps and adult leg counts. Monitoring by District staff may occur as often as 3-4 times per week during the summer irrigation (May 1st-July 31st) and fall flood up (August 1st-October15th). If temperatures are above average beyond October 15, District staff may continue to require access to the Refuge for monitoring.

Light and carbon dioxide traps are used to capture adult mosquitoes for monitoring purposes. Dip counts are used to estimate the numbers of immature mosquitoes and to determine the need for larval mosquito control. The dipper method entails using a long-handled ladle (ca 500 ml) called a dipper to collect water samples from possible mosquito sources. Captured immature mosquitoes are identified taxonomically as precisely as possible. All Refuge units supporting wetlands or irrigated land potentially may be monitored using the dipper method. However, units supporting managed wetlands would be targeted. Sampling locations for larvae may include wetland margins and shorelines and riparian habitats for adults.

As provided for in the MOU, the monitoring activities described above are conducted under a Special Use Permit (SUP) that the Refuge intends to continue issuing annually to the District.

1. Larval Mosquito Thresholds

Guidelines for control of immature or larval mosquitoes follow integrated pest management principles and are defined in a District Mosquito and Mosquito-Borne Disease Management Plan (Appendix I), available on their website (http://www.fightthebite.net) (Boyce 2005). In keeping with the MOU, the District requests annual approval from the Refuge to control mosquitoes by treating areas where larval stages of *Culex tarsalis*, *Aedes melanimon, A. nigromaculis, Anopheles freeborni* or other *Aedes* spp. may exceed thresholds. According to the District Management Plan, the threshold for initiating a larval control response will be a density of 0.1 mosquito larvae per 350-ml dipper of water for all species.

2. Adult Mosquito Thresholds

The District Management Plan defines criteria for five possible levels of adult mosquito activity and control responses (Appendix II). The thresholds for Level 1 (Standard or

Routine) adult mosquito control are 10 *Culex tarsalis* female mosquitoes per light trap night or 100 per CO_2 baited trap per night. For *Aedes* spp. the thresholds for Level 1 control are 50 female mosquitoes per light trap night or 150 per CO_2 baited trap per night. Under Level I, the thresholds for landing (leg) count collections are exceeded when two or more *Aedes* or *Ocheloratus* spp. land on an individual during a one-minute interval. Level 1 control in the District Management Plan is consistent with Level 1 (Normal Season) in the California Department of Health Services Mosquito-Borne Virus Surveillance and Response Plan (CA Dept. of Heath Services 2004).

The District Management Plan calls for a Level 2 control response when a mosquito-borne virus is confirmed from a dead bird or mosquito pool within District boundaries. The threshold for levels 2-5 adult control is 10 *Culex tarsalis* or *Cx. Pipiens* female mosquitoes per light trap night or 25 per CO_2 baited trap per night. For *Aedes* spp. the thresholds for Level 2-5 control responses are 25 female mosquitoes per light trap night or 50 per CO_2 baited trap per night. Level 2 control in the District Management Plan is equivalent to Level 2 (Epidemic Conditions) in California Department of Health Services Mosquito-Borne Virus Surveillance and Response Plan (CA Dept. of Heath Services 2004). According to the District Management Plan, levels 2-5 adult treatment thresholds remain at the reduced level until control activities are terminated for the mosquito season. The thresholds used by the District are based on historical monitoring that indicate all mosquitoes have the potential to transmit a wide range of diseases. Thresholds also minimizes annoyance levels to nearby communities from adult mosquitoes.

IV. Surveillance of Mosquito-Borne Disease

Vectorborne disease surveillance and associated health threat determinations are made by the California Vectorborne Disease Surveillance System (CVDS), a cooperative project of the Mosquito and Vector Control Association of California, the California Department of Health Services (CDHS), and the University of California at Davis. The UC Davis Center for Vectorborne Diseases (CVEC) analyzes samples collected from mosquito pools, sentinel chickens and dead birds and publishes results in the California Arbovirus Surveillance Bulletins. The samples are collected by the District.

The District arbovirus surveillance program includes testing of mosquito populations, sentinel chickens and wild birds for WEE, SLE and WNV. The information generated by the encephalitis program provides an early indication of local arboviral activity. Small populations of mosquitoes from sites that have a history of disease activity are sampled and tested by the mosquito abatement district. Carbon dioxide traps attract and capture mosquitoes which are subsequently identified, sorted and grouped into pools. The pools are sent to the CVEC where they are tested for encephalitis viruses. Mosquitoes are collected annually from March to November.

As part of monitoring conducted by the District for the presence of mosquito borne public health diseases, a sentinel chicken flock is maintained on private property adjacent to the Refuge Headquarters Unit. Service Region 1 policies prevent placement of sentinel chicken flocks on wildlife refuges because of the risk of avian diseases passing from chickens to migratory birds. Sentinel chickens are exposed to the environment and to mosquitoes moving through the area that may choose to feed on them. Regular blood samples are periodically taken from the chickens to detect any mosquito-vector pathogen activity. Once the flock exhibits positive viral titers and sero-conversion occurs, the California Department of Health Services is alerted to the potential threat to public health due to mosquito borne diseases.

For approximately the last ten years, Refuge staff and the District have conducted a collaborative research effort to collect blood samples from resident and migratory birds captured on the Refuge. The wild bird sera samples are processed and tested for the presence of WEE, SLE and WNV virus antibodies at the District laboratory. These cooperative efforts also provide the Service with important data regarding the status of migratory bird populations occurring on the Refuge.

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Reporting

1. The District will notify the Refuge Manager in the event of detection of virus activity within or near the Refuge and the method of disease surveillance yielding positive results.

2. Refuge staff will participate in collections of wild bird sera for testing and will monitor any unusual bird die-offs that may be caused by WNV or other diseases. Wild bird mortality due to WNV can provide an early warning of the risk of transmission to the public via mosquitoes.

V. Treatment Options

Mosquito control at the Refuge follows an ordered succession, using nonchemical treatments first (i.e., wetland and water delivery system design, water control strategies, vegetation management, mosquitofish, guppies, and other biological agents, etc.), resorting to chemical treatment only when necessary, as determined through standard mosquito monitoring procedures. Among chemical treatments, adulticides will be used as a last resort. Refuge staff work with the District to minimize production of mosquitoes on the Refuge by means of habitat management and biological controls and are mindful of abiotic sources of mosquito production (e.g., tanks, buckets, equipment holding water) and promptly eliminate them if discovered.

Habitat Management

Refuge habitat management techniques that support mosquito abatement consist of: (1) design of managed wetlands, (2) efficient water management, and (3) physical manipulation of vegetation. These are mostly preventative measures to eliminate or reduce mosquito breeding habitat or conditions before it develops. If habitats were managed entirely for mosquito abatement, wildlife habitat values would be compromised. Therefore, Refuge staff strive to incorporate management techniques for mosquito abatement into ongoing wildlife habitat efforts. In an officially determined health emergency, mosquito abatement would become a higher priority than habitat management.

Wetland Design and Water Management

Water management techniques for minimizing mosquito production include timing and duration of flooding, the speed at which individual units are flooded up, irrigated, or drawn down and the stability of water levels. The Refuge is only able to conduct efficient water management on managed wetlands impoundments with pumps and water control structures where they can be manipulated to reduce mosquito production. These occur on the Beach Lake, Headquarters, and South Stone Lake units. Elements incorporated into the design of these wetlands that promote minimizing mosquito breeding include contouring of wetland margins, construction of drainage swales, and sizing of water control structures for relatively rapid flood up and de-watering. Steeper sloped wetlands support narrower perimeter margins where warm, shallow, vegetated conditions provide optimal breeding habitat for many species of mosquitoes.

Managed permanent and summer water wetlands produce the fewest numbers of mosquitoes and pose the least concern for the District. Permanent wetlands are flooded to a depth of approximately three feet which minimizes their use by floodwater mosquitoes and encourages abundant populations of mosquito predators (e.g., fish, dragonflies). Deep, open water provides water circulation and generates wave action that reduces micro-habitats suitable for mosquito breeding.

Seasonal wetland impoundments with water control structures are managed to provide wildlife habitat while minimizing the potential for mosquito production. Water delivery infrastructure is sized for rapid flood up, irrigations, and draw down, providing at least two means by which mosquito production is reduced. Slow irrigations, especially over large units, usually result in multiple hatches of adult mosquitoes. For example, if it takes five days to inundate a unit, a new hatch of eggs (i.e., typically *Aedes melanimon*) could be produced every day resulting in five separate cohorts of larvae/pupae and subsequent adult mosquitoes emerging over a five day period. The alternative is to flood up rapidly, covering the entire unit within one day if possible. This will not reduce the overall amount of mosquitoes produced but it will result in a more synchronous egg hatch and adult emergence. This will facilitate a more efficient control effort, requiring fewer applications for the same objective.

The timing of the fall flood up can substantially affect both mosquito production and wildlife habitat values. Delaying the initial fall flood up also delays associated initial mosquito production and may reduce the need for mosquito abatement if temperatures have already dropped sufficiently to discourage mosquitoes. Historically, Sacramento Valley wetlands flooded naturally and much later, in the fall or early winter, based largely on the Sacramento River overflowing its banks. However, current managed wetland flooding regimes are dictated largely by water availability through irrigation districts and the need to provide wetlands for early migrating waterbirds during August-September. For example, providing roosting habitat for sandhill cranes is a priority at the Refuge which requires flooding up by mid-September, when they first arrive in the valley. Though this practice conflicts with District abatement recommendations, sandhill crane habitat considerations are given precedence over mosquito control. In the event of a threat to public health and/or unusually warm fall temperatures that would encourage mosquito production, delaying fall flood up remains an option.

Effective communication between Refuge staff and the District remains a critical requirement for coordinated mosquito management. In addition to submitting an annual summary of planned water management to the District, Refuge staff provide advance notifications of flood ups and irrigations so mosquito monitoring and possible treatments can be scheduled at an optimal time. Such notifications can result in applications of adulticides not being required since the District then has an opportunity to control larval populations, thus controlling adult emergence rates. For example, the Refuge attempts to flood wetlands early in the week so that mosquito hatches do not occur over the weekend when District staff are not in the field.

Physical Manipulation of Vegetation

Vegetative structure in wetlands provides habitat features that generally favor mosquito production. The benefits of vegetation include egg-laying sites, protection from the elements, and escape cover. The literature suggests that reduction of vegetation by burning or mowing (Batzer and Resh 1992) can reduce mosquito production significantly.

Mowing, herbicide applications, disking, or burning are the most common methods of reducing accumulations of vegetation in wetlands on the Refuge. Depending on the extent of vegetation they support, managed wetlands may be manipulated during the dry season to support a target mix of open water to emergent vegetation: approximately 50% vegetation to 50% open water. These manipulations improve wildlife habitat by promoting wetland plants of more food value for migratory waterfowl, increasing edge habitat, and the overall openness of wetland units, making them more attractive to shorebirds and species such northern pintail, green-winged teal, and sandhill crane. These treatments also reduce the extent of mosquito breeding habitat and improve accessibility to mosquito larvae by mosquito fish and other natural predators. Seasonal wetlands lacking in water control structures cannot be as easily managed but wetland perimeters may be mowed or disced both to improve conditions for mosquito fish and as well as to increase their value for waterbirds.

Major portions of Refuge waterways (e.g., South Stone Lake, SP Cut) have been invaded by the invasive aquatic plants, water hyacinth and Brazilian elodea (Egeria densa). Left unchecked, these continuous floating or submerged mats of vegetation can encourage mosquito production by providing harborage from predation, concentration of organic foods, and interference with wave action and water circulation. The Refuge was a founding member and has the lead role in the Stone Lakes Basin Water Hyacinth Control Program, along with the District and the Sacramento Regional County Sanitation District. This program contributes to both mosquito abatement and wildlife habitat improvement goals.
In summary, habitat management techniques promoting mosquito abatement include:

- Construct wetland impoundments with appropriate slopes and adequate water management capacities
- Flood up/irrigate quickly to discourage multiple hatches

- Maintain a depth of 2-3 feet of water in permanent wetlands
- Control emergent vegetation to maintain 50 percent open water in managed wetlands
- Disc/mow pond perimeters in seasonal wetlands to maintain open water and access by fish
- Control invasive aquatic weeds
- Notify the District of planned flood up/irrigation events

Biological Controls

Reducing production of mosquitoes in a wetland ecosystem is partially dependant upon maintaining a diversity of habitats that support various predators and parasitic species that can then control mosquito populations. Predators and parasites can take sizable numbers of mosquitoes but if conditions support the rapid development of mosquitoes, then natural predation can be augmented by the addition of insectivorous (insect eating) fishes. The District has introduced three species of insectivorous fish to the Refuge, mosquito fish (*Gambusia affinis*), guppies (*Poecilia reticulata*) and the native threespine stickleback (*Gasterosteus aculeatus*).

Mosquitofish

Mosquitofish have played an active role in mosquito larvae control at refuges within California's Sacramento Valley over the last twenty years. Mosquitofish exhibit a tremendous tolerance for a wide range of water temperatures. Previously acclimated fish may tolerate minimum and maximum temperatures of 33° and 104°F (0.6° and 40°C), although sudden drastic changes of temperature are often lethal. Preferred temperatures appear to lie between 77° and 86°F (25° and 30°C). When surface water temperatures approach higher lethal limits, mosquitofish usually swim down to cooler water strata. Conversely, in the cooler seasons mosquitofish will move into shoal areas to reach the sun-warmed shallow waters. Other environmental factors that influence mosquito fish survival include densities of mosquito larvae, aquatic vegetation, availability of alternative forage organisms, presence of predaceous bird and fish populations, water depth and flow patterns, and several water quality criteria. For the fish to be effective, there must be no limitations to their normal distribution, rapid reproduction, and population recruitment. In general, mosquitofish are stocked in very small numbers because they quickly reproduce to the maximum population levels that a particular habitat may sustain.

Mosquitofish are transported by tanker truck directly to mosquito sources. Primary stockings of fish in semi-permanent wetlands are usually conducted at a minimal initial rate of 0.1 lb per acre. When necessary, these applications are augmented up to 1.0 lb per acre, based on larval dipping data. The District has stocked Refuge waters with mosquitofish every year since 1996. Most of the mosquitofish have been planted in the Beach Lake Unit, but some have also been placed in small ponds on the Sun River and Beach Lake properties.

Guppies and Threespine Sticklebacks

The District is evaluating the use of guppies and threespine sticklebacks for mosquito larvae control. Literature suggests that guppies will do an excellent job of controlling mosquitoes during the summer months but will not survive the cold winter months. Using this species in areas that are prone to winter flooding will ensure that these fish will not impact threatened or endangered species occurring within the floodplain. Threespine sticklebacks prefer to feed on benthic organisms rather than on the surface where mosquito larvae are found, but where the benthic community is insufficient, the Sticklebacks will expand their feeding range to the surface. Sticklebacks were only planted at the Refuge in 2001, while guppies were used in 2002 and 2003.

Chemical Controls

Larvicides/Pupacides

The District proposes to control mosquitoes by treating areas infested with larval stages of *Culex tarsalis*; *Aedes melanimon, A. nigromaculis, Anopheles freeborni* and *Aedes* spp.

The District would use the biological larvicides *Bacillus thuringiensis isrealensis* (Bti) and *Bacillus sphaericus* (Bsp) and the insect growth inhibitor methoprene. Use of the petroleum distillate Golden Bear (GB1111) as a pupacide was discontinued after 2000 and has been replaced with the monomolecular film Agnique. These treatments would be applied via ground methods. Based on December 2005 Service Delegation of Approval Authority for the California Nevada Operations (CNO) area, refuge managers now have authority to approve use of Bti, Bsp, methoprene, and Agnique.

Bti is a microbial insect pathogen used to control larval stages of mosquitoes and black flies. It is a naturally occurring anaerobic spore forming bacteria that is mass produced using modern fermentation technology. Bti produces protein endotoxins that are activated in the alkaline mid-gut of insect species and subsequently bind to protein specific receptors of susceptible insect species resulting in the lethal response (Lacey and Mulla 1990). Bti must therefore be ingested by the target insect to be effective. It is most effective on younger mosquito larval instars but does not affect pupae or adult mosquitoes. The District prefers to use Bti because of the low impacts to the environment and non-target organisms and its effectiveness in reducing the numbers of mosquito larvae. The Bti formulations Vectobac 12AS or Vectobac G would be employed at the Refuge by the District. Bti may be applied at the Refuge between March and October.

Like Bti, Bsp is a microbial insect pathogen with a similar mode of action (Walton 1998). Formulated Bsp products used as mosquito larvicides consist of bacterial spores and protein endotoxins. The granular formulation of Bsp, Vectolex CG, would be applied by the District. Both Bti and Bsp may be applied as a spot treatment to small areas or broadcast over larger areas. Use of Bsp is permitted between June 1 and September 30 and applications would likely be made within 7-10 days of initial flooding to control third and fourth instar larva.

Methoprene is a synthetic insect growth regulator (IGR) that mimics juvenile hormones (Tomlin 1994). It interferes with the insect's maturation stages preventing the insect from transforming into the adult stage, thereby precluding reproduction. Methoprene is a contact insecticide that does not need to be ingested. It is most effective on early larval instars but does not affect pupae or adult mosquitoes (ETN 1996a). Treated larvae will pupate, but will not emerge as adults. The District proposes to use the formulated methoprene product Altosid in pellets or A.L.L. Growth Regulator. Use of methoprene is permitted between June 1 and September 30.

The monomolecular film, Agnique, reduces water surface tension. This interferes with larval orientation at the air-water interface and/or increases wetting tracheal surfaces, thus suffocating the organism. As the film spreads over the water surface, it tends to concentrate mosquito pupae, which may increase mortality from crowding stress (Dale and Hulsman 1990). Use of Agnique is permitted between June 1 and September 30.

Area Subject to Larvicides
Applications of larvicides may occur in managed permanent wetlands (106 acres), irrigated pastures (490 acres) and occasionally perennial wetlands (193 acres), totaling approximately 790 acres. The shorelines of open water areas may be treated. In addition, the District will treat ditches, culverts and low areas not classified as wetlands.

As a result of IPM practices and cooperation between the Refuge and the District, larval control applications on the Refuge have been limited to small acreages during any single treatment (less than five acres). The total Refuge acreage that may be treated varies with rainfall conditions each year. During drought years mosquito populations tend to be low, and during wet years mosquito populations tend to be high. From 2000-2004, the range in total acreage treated varied from a low of 104 acres in 2000 to a high of 477 acres in 2004. The majority of the treatments occur from August to October, but in some years applications of Bti have begun as early as March and have extended into November.

Adulticides

If efforts to control immature mosquitoes fail to prevent the adult mosquito population from exceeding thresholds, and a documented historical or current health threat exists, the District proposes to treat infested areas with a mosquito adulticide. The District has requested annual approval for use of liquid formulations of synthetic pyrethrins, such as Pyrenone 25-5 or Pyrocide 7338. The District also requests and has received approval for use of the adulticide Trumpet (Naled) but it has not been used on the Refuge to date. Use of all adulticides is limited to June 1 through September 30, with a possible extension if unusually hot weather occurs together with a documented public health threat. Based on the December 2005 Service Delegation of Approval Authority for the CNO area, refuge managers have authority to approve use of pyrethrins for controlling mosquitoes near facilities used by staff and visitors and in terrestrial sites (not aquatic sites or wetlands). Approval of use of pyrethrins in aquatic or wetland settings or Trumpet necessitates submittal of Pesticide Use Proposals to the CNO and Washington offices.

Pyrethrins are non-systemic contact poisons which quickly penetrate the nerve system of the insect and cause paralysis and subsequent death (ETN 1994, Tomlin 1994). A few minutes after application, the insect cannot move or fly away. But, a “knockdown dose” does not mean a killing dose. Pyrethrins are swiftly detoxified by enzymes in the insect. Thus, some pests will recover. To delay the enzyme action so a lethal dose is assured, commercial products are formulated with synergists such as piperonyl butoxide, which inhibit detoxification (Tomlin, 1994). Trumpet (Naled) is a non-systemic, broad-spectrum organophosphate insecticide which affects the nervous system of adult mosquitoes and other insects by cholinesterase inhibition. When treatments occur, Pyrethrins and Trumpet, would be applied as an ultra-low volume (ULV) mist by ground. To minimize pesticide drift, dispersing vehicles follow routes on existing roads set up to fog downwind or outside buffers of 300 feet from areas supporting listed or proposed special status species. All chemical applications occur when wind speeds are between 2 and 8 mph.

Between 1994 when then Refuge was established and 2004, adult mosquito applications only occurred once in 1998 to five acres (0.09 gallons of Scourge) and once in 1999 to four acres (0.05 gallons of Pyrocide 7338). Both treatments were ULV ground applications at the same location, a drainage channel on the Headquarters Unit. That adulticides were utilized so infrequently, attests to the level of cooperation between District and Refuge who initiated water management and larval control measures to discourage mosquito production and adult emergence.

In 2005, West Nile Virus (WNV) became established in Sacramento and Yolo counties, triggering more aggressive and widespread mosquito control efforts. In August of 2005 the number of human WNV cases and rate of infected adult mosquitoes were so high that SYMVCD initiated aerial applications of pyrethrin over significant portions of Sacramento County. The Refuge received ultra-low volume (ULV) ground applications of pyrethrin on 16 occasions between July 28 and October 12, 2005. As of August 18, 2006, 16 human cases of WNV have been documented in Sacramento and Yolo counties (Table 1) and the Refuge has had adulticides applied 12 times beginning on June 27.

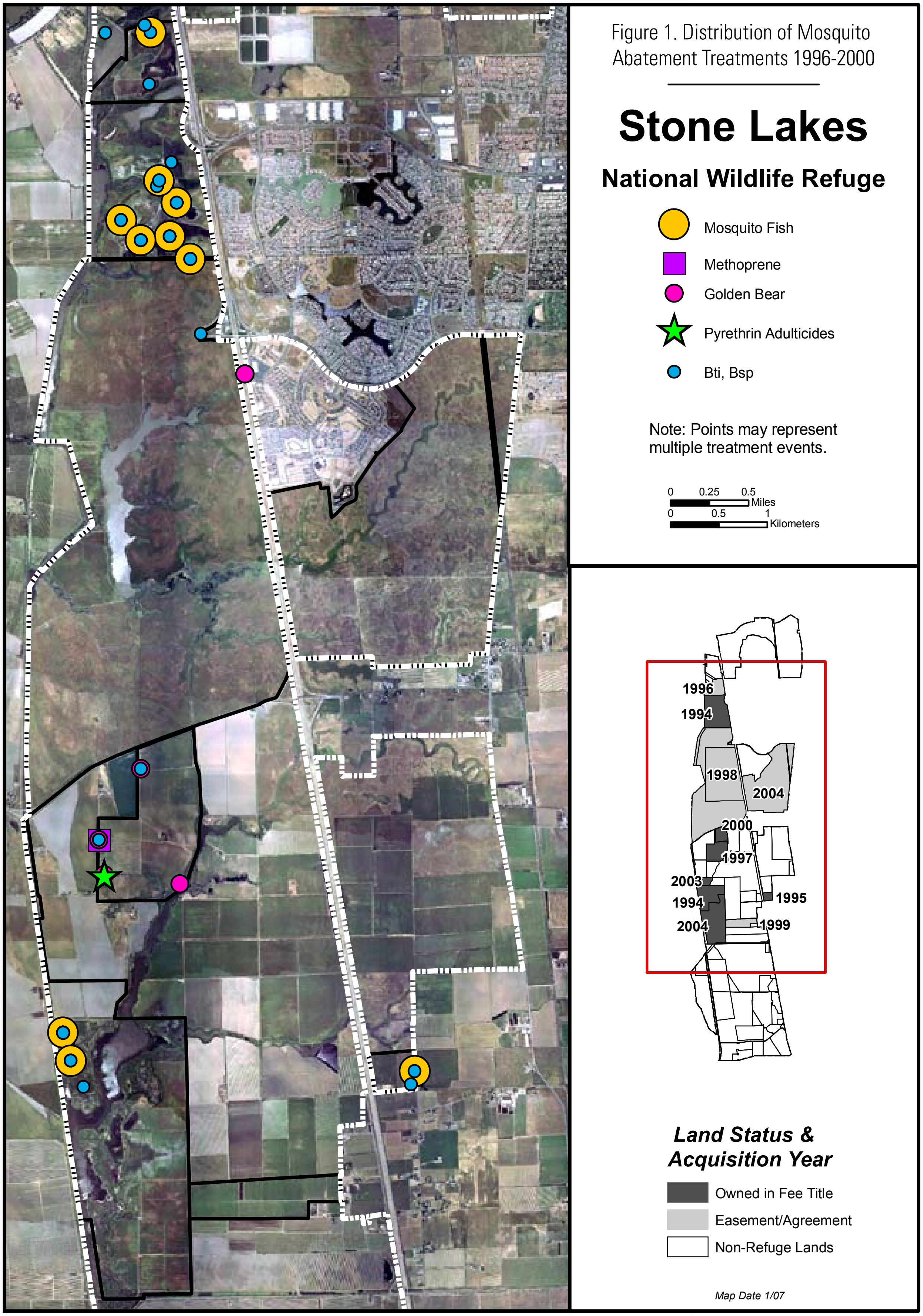


Figure 1. Distribution of Mosquito Abatement Treatments 1996-2000

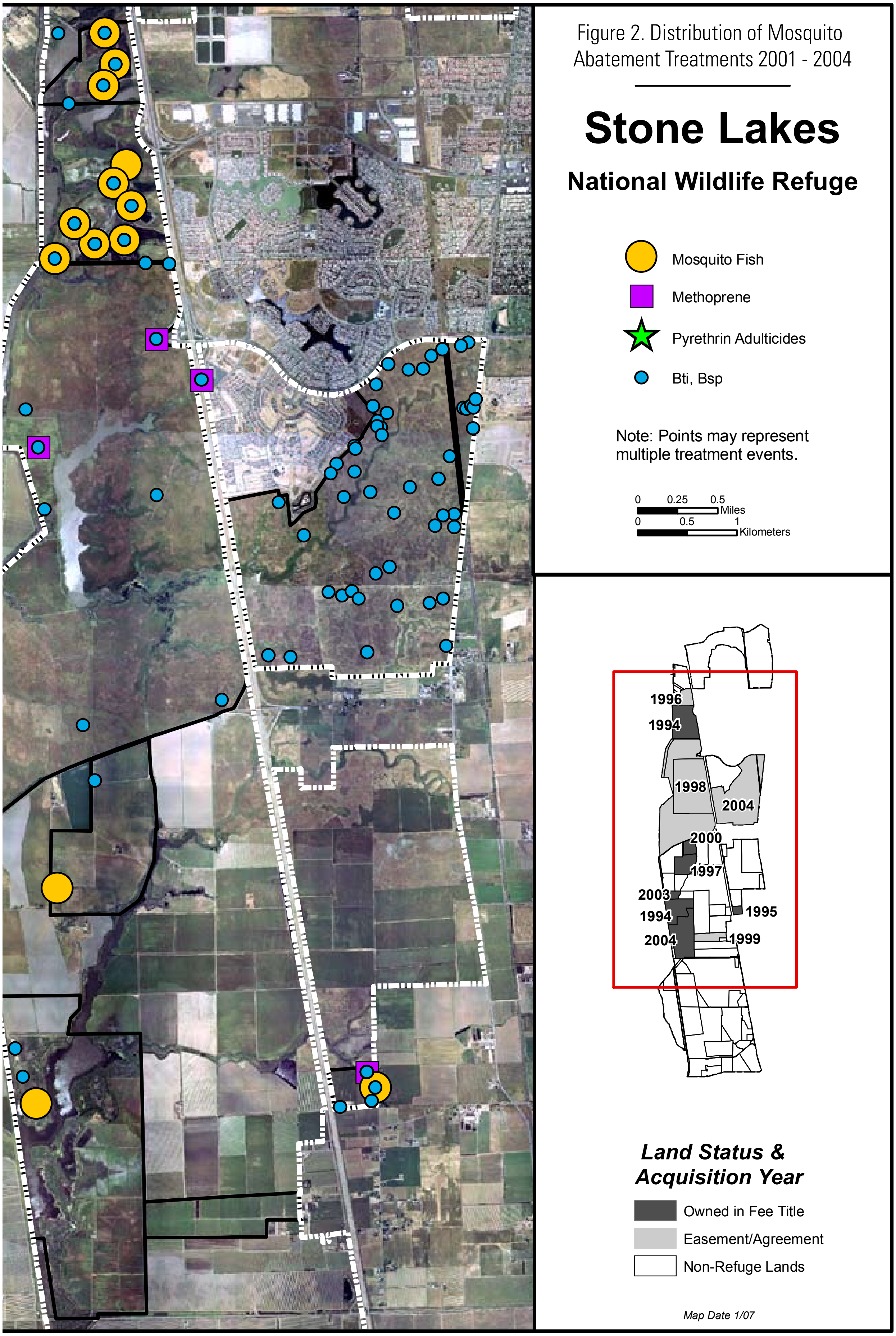
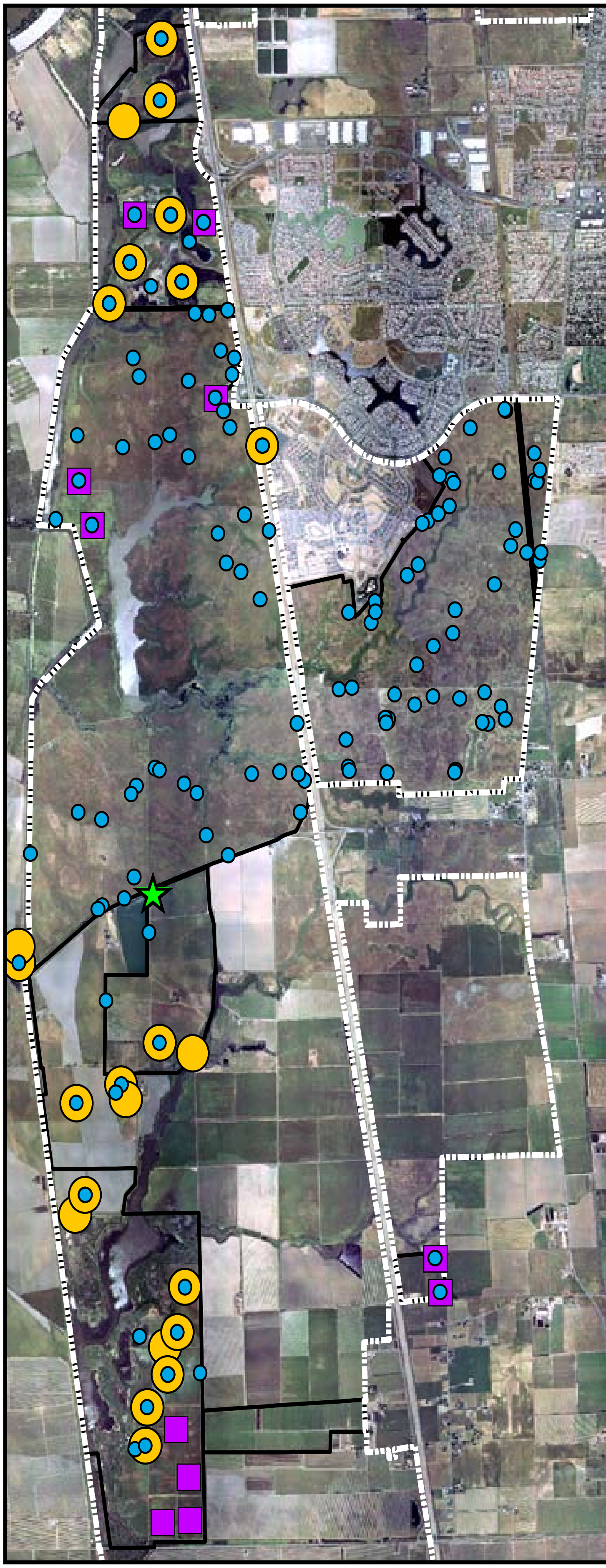


Figure 2. Distribution of Mosquito Abatement Treatments 2001 - 2004

Figure 3. Distribution of 2005 Mosquito Abatement Treatments

Stone Lakes

National Wildlife Refuge



Mosquito Fish

Methoprene

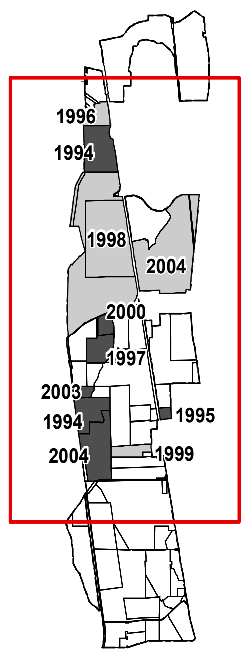
Pyrethrin Adulticides

Bti, Bsp

Note: Points may represent multiple treatment events.

0 0.25 0.5 Miles

0 0.5 1 Kilometers



Land Status & Acquisition Year

Owned in Fee Title

Easement/Agreement

Non-Refuge Lands

Map Date 1/07

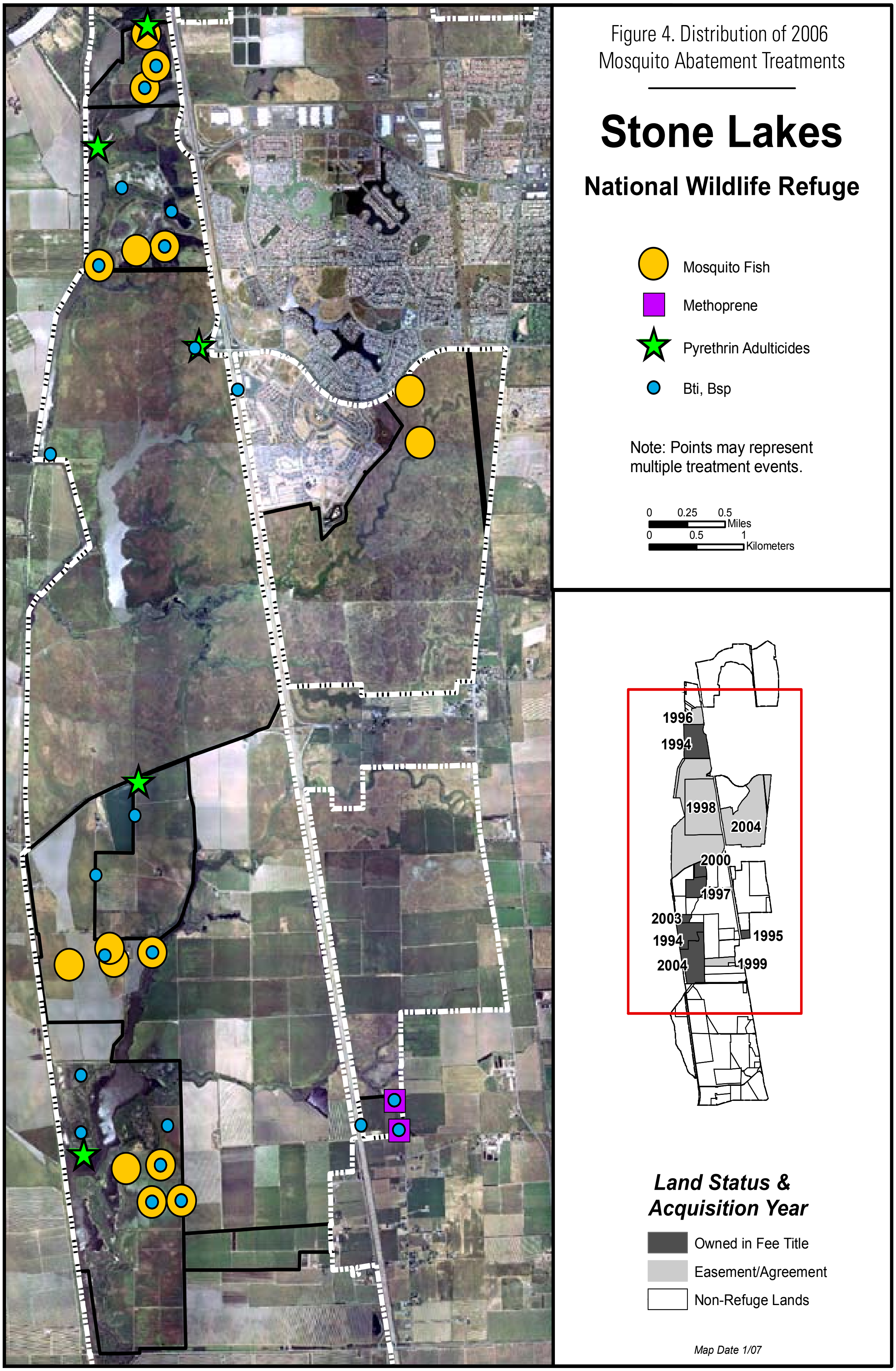


Figure 4. Distribution of 2006 Mosquito Abatement Treatments

VI. Toxicity and Effects to Non-Target Organisms

The dominant impact of mosquito control will relate to the toxicity and effects of the treatments on non-target organisms. The possible effects of the larvicides *Bacillus* spp. and methoprene, the pupacide Agnique, and the adulticides will be discussed separately.

Larvicides

Bacillus thuringiensis isrealensis (Bti)

Bti has practically no acute or chronic toxicity to mammals, birds, fish, or vascular plants (U.S. EPA 1998). Extensive acute toxicity studies indicated that Bti is virtually innocuous to mammals (Siegel and Shadduck 1992). These studies exposed a variety of mammalian species to Bti at moderate to high doses and no pathological symptoms, disease, or mortality were observed. Laboratory acute toxicity studies indicated that the active ingredient of Bti formulated products is not acutely toxic to fish, amphibians or crustacaceans (Brown et al. 2002, Brown et al. 2000, Garcia et al. 1980, Lee and Scott 1989, and Wipfli et al. 1994). However, other ingredients in formulated Bti products are potentially toxic. The acute toxicity response of fish exposed to the formulated Bti product Teknar® HPD was attributed to xylene (Fortin et al. 1986, Wipfli et al. 1994). Field studies indicated no acute toxicity to several fish species exposed to Bti (Merritt et al. 1989, Jackson et al. 2002); no detectable adverse effects to breeding red-winged blackbirds using and nesting in Bti treated areas (Niemi et al. 1999, Hanowski 1997); and no detectable adverse effects to tadpole shrimp 48 hours post Bti treatment (Dritz et al. 2001).

In addition to mosquitoes (Family Culicidae), Bti affects some other members of the suborder Nematocera within the order Diptera. Also affected are members of the Family Simuliidae (black flies) and some chironomids midge larvae (Boisvert and Boisvert 2000, Garcia et al. 1980). The most commonly observed Bti effects to non-target organisms were to larvae of some chironomids in laboratory settings when exposed to relatively high doses (Boisvert and Boisvert 2000, Lacey and Mulla 1990, Miura et al. 1980). In field studies, effects to target and susceptible nontarget invertebrates have been variable and difficult to interpret. Field study results are apparently dependent on the number, frequency, rate and aerial extent of Bti applications; the Bti formulation used; the sample type (e.g., benthic, water column or drift); the sampling interval (e.g., from 48 hrs to one or more years after treatment); the habitat type (e.g., lentic or lotic); the biotic (e.g., aquatic communities), and abiotic factors (e.g., suspended organic matter or other suspended substrates, temperature, water depth); the mode of feeding (e.g., filter feeder, predator, scraper or gatherer); the larval development stage and larval density (Ali 1981, Boisvert and Boisvert 2000, Lacey and Mulla, 1990). Bti activity against target and susceptible nontarget invertebrates is also related to Bti persistence and environmental fate which are in turn affected by the factors associated with field study results (Dupont and Boisvert 1986, Mulla 1992). Simulated field studies resulted in the suppression of two unicellular algae species, *Closterium* sp. and *Chlorella* sp. resulting in secondary effects to turbidity and dissolved oxygen of aquatic habitats, with potential trophic effects (Su and Mulla 1999). For these reasons, Bti effects to target and susceptible nontarget organisms, and potential indirect trophic impacts in the field are difficult to predict.

Bacillus sphaericus (Bsp)

Bsp has slight to practically no acute mammalian toxicity, practically no acute avian toxicity, slight to practically no acute fish toxicity, and slight aquatic invertebrate toxicity (USFWS 1984, and FCCMC 1998). Insecticidal activity may persist longer than 20 days because Bsp can reproduce and sporulate in larval cadavers (Becker et al, 1995) and can retain its larvicidal properties after passing through the gut of a mosquito. Bsp is insoluble in water. Spores and toxin become suspended in the water column and retain insecticidal activity in water with high organic matter content and suspended solids. Because Bsp is a more recently developed larvicide than Bti, there are fewer studies that have examined the non-target effects of this pesticide. The data available, however, indicate a high degree of specificity of Bsp for mosquitoes, with no demonstrated toxicity to chironomid larvae at any mosquito control application rate (Mulla, 1984, Ali, 1986, Lacey, 1990, and Rodcharoen, 1991). Therefore risks to sensitive wildlife resources resulting from direct exposure to a single Bsp application and indirect food chain effects are expected to be negligible. However, the ability for a population to re-colonize a wetland following multiple larvicide treatments would depend on the intensity and frequency of applications at different spatial scales.

Agnique (Monomolecular film)

Monomolecular film has practically no acute mammalian or avian toxicity, and slight acute fish toxicity (USEPA 2000, USFWS 1984). The risk quotient for mammals is well below the EPA endangered species level of concern (LOC) indicating negligible risk resulting from direct exposure, Table 2 (Urban and Cook 1986). Risk quotients for birds and fish exceed EPA endangered species LOCs indicating a hazard to those taxa resulting from direct exposure. Risk to fish is limited by the insolubility of monomolecular film in water. Monomolecular film is insoluble in water, average persistence in the environment is 5 to 14 days. Indirect effects to animals dependent on invertebrate food resources are possible resulting from a reduction of those resources caused by monomolecular film. The magnitude of the impact would depend on the aerial extent of the treatment, the number of treatments, treatment frequency, and the location of the treatment relative to the areas used by invertebrate feeding animals.

Table 2. Monomolecular film risk quotients.

Animal	Acute toxicity (ppm)	EEC (ppm)	RQ	LOC (ES)
bird	> 5000 (8 D LC 50)	850 (short grass)	0.2	0.1
fish	98 (96 hr LC 50)	2600 (6" water)	26.5	0.05
mammal	>20,000 (LD 50)	850 (short grass)	0.004	0.1

EEC calculated using a rate of 0.5 gal/ac (3.6 lbs ai/ac)
LD 50 for mammals converted to 1 Day LC50 using a conversion factor of 0.1 for RQ calculation

Methoprene

Methoprene has moderate acute fish toxicity, slight acute avian toxicity, and practically no acute mammalian toxicity (USEPA 2000, and USFWS 1984). In mallard ducks, dietary concentrations of 30 parts per million (ppm) caused some reproductive impairment (USEPA 1991). This figure exceeds the estimated environmental concentration by a factor 10 (Table 1). Methoprene residues have been observed to bioconcentrate in fish and crayfish by factors of 457 and 75, respectively (USEPA 1991). Up to 95 percent of the residue in fish was excreted within 14 days (USEPA 1991). Risk quotients for birds, fish and mammals are below EPA levels of concern for endangered species indicating negligible risk to those taxa resulting from direct exposure using maximum labeled rates for mosquito control (Table 3) (Urban et al. 1986). In field studies no detectable adverse effects to breeding red-winged blackbirds using and nesting in areas treated with methoprene were observed (Niemi et al. 1999).

Methoprene affects terrestrial and aquatic invertebrates and is used to control fleas, sciarid flies in mushroom houses; cigarette beetles and tobacco moths in stored tobacco; Pharaoh's ants; leaf miners in glasshouses; and midges (Tomlin 1994). Methoprene may also be fed to livestock in a premix food supplement for control of hornfly (WHO undated). Methoprene is

Table 3. Risk assessment for Methoprene.

Animal	Acute Tox (ppm)	EEC (ppm)	RQ	LOC (ES)
Bird	> 4640 (8 D LC 50)	3.0 (short grass)	0.0006	0.1
Fish	0.4 (96 hr LC 50)	0.01 (6 inches)	0.025	0.05
Mammal	> 34,000 (LD 50)	3.0 (short grass)	0.00001	0.1

EEC calculated using a rate of 0.013 lbs ai/ac (1.0 fluid oz/ac Altosid 20 % methoprene)
LD 50 for mammals converted to 1 Day LC50 using a conversion factor of 0.1 for RQ calculation

highly toxic to aquatic invertebrates with a 48 hour EC50 (the concentration of a compound where 50 percent of its effect is observed) of 0.89 ppm for *Daphnia magna* (USEPA 1991). Laboratory studies show that methoprene is acutely toxic to chironomids, cladocerans, and some decapods (Horst and Walker 1999, Celestial and McKenney 1994, McKenney and Celestial 1996, Chu et al. 1997). In field studies, significant declines of aquatic invertebrate, mollusk and crustacean populations have been directly correlated to methoprene treatments for mosquito control (Breaud et al. 1977, Miura and Takahashi 1973, Niemi et al. 1999, and Hershey et al. 1998).

Methoprene has a ten day half life in soil, a photolysis half life of ten hours, and solubility in water is 2 ppm (Zoecon 2000). Degradation in aqueous systems is caused by microbial activity and photolysis (USEPA 1991). Degradation rates are roughly equal in freshwater and saltwater systems and are positively correlated to temperature (USEPA 1991).

Adulticides

There are only two general classes of mosquito adulticides, organophosphates and pyrethroids. The pyrethroids include both natural products called pyrethrins and synthetic molecules that mimic the natural pyrethrins, such as permethrin, resmethrin, and sumithrin. One organophosphate, Trumpet (Naled), is approved for use at the Refuge in the past but not applied to date. The two pyrethroid products approved for use at the Refuge, Pyrenone 25-5 and Pyrocide 7336 are both synthetic pyrethrins.

In general, pyrethroids have lower toxicity to terrestrial vertebrates than organophosphates. Although not toxic to birds and mammals, pyrethroids are very toxic to fish and aquatic invertebrates (Anderson 1989, Siegfried 1993, Milam et al. 2000). The actual toxicity of pyrethroids in aquatic habitats, however, is less than may be anticipated because of the propensity of these pesticides to adsorb organic particles in water (Hill et al. 1994). Pyrethrins are toxic to all invertebrates, but the method of application via ultra-low volume atomizer limits toxicity and contact with non-targets. To minimize pesticide drift, applications would take place during the evening hours, when wind speeds are reduced and temperatures decreased; this is also the period when mosquito activity is the greatest. Naled is a fast acting, nonsystemic contact and stomach organophosphate insecticide used to control aphids, mites, flies, and mosquitoes. Naled is highly to moderately toxic via the oral route. It is moderately toxic through skin exposure, may cause skin rashes and skin sensitization and may be corrosive to the skin and eyes. Naled is highly to moderately toxic to birds. The reported acute oral LD50 (lethal dose 50, the dose of a substance which is fatal to 50% of the test animals) for naled is 52 mg/kg in mallard ducks, 65 mg/kg in sharp-tailed grouse, 36-50 mg/kg in Canadian geese, 120 mg/kg in ring-neck pheasants. Naled is highly to moderately toxic to fish and may be very highly toxic to aquatic invertebrate species (ETN 1996). However, Trumpet (Naled) is practically nonpersistent in the environment, with reported field half-lives of less than 1 day. It is not strongly bound to soils and is rapidly broken down if wet. Soil microorganisms break down most of the naled in the soil. It therefore should not present a hazard to groundwater (ETN 1996).

Threatened and Endangered Species

The Refuge provides potential habitat for the following federally-listed species: giant garter snake, valley elderberry longhorn beetle, vernal pool tadpole, and vernal pool fairy shrimp. Potential impacts to these species from mosquito control activities were addressed in a number of previous Intra-Service Section 7 Consultations conducted with the Sacramento Fish and Wildlife Office (SFWO):

March 27, 1995: The SFWO concurred with the determination that use of the bacterium *Bacillus thuringiensis israelensis* (Bti) and Altocid® (methoprene) for mosquito control at Stone Lakes NWR is not likely to adversely affect the vernal pool fairy shrimp, vernal pool tadpole shrimp, giant garter snake, valley elderberry longhorn beetle, Sacramento splittail, and delta smelt (SFWO file: 1-1-95-I-0680).

January 9, 1997: The SFWO concurred with the determination that the use of the bacterium *Bacillus sphaericus* for mosquito control, is not likely to adversely affect the vernal pool fairy shrimp, vernal pool tadpole shrimp, giant garter snake, valley elderberry longhorn beetle, delta smelt, or Sacramento splittail at the Stone Lakes NWR (SFWO file: 1-1-96-I-0639).

January 31, 2001: The SFWO concurred that pest management activities at the Refuge are not likely to jeopardize the giant garter snake, valley elderberry longhorn beetle, vernal pool tadpole shrimp, or vernal pool fairy shrimp. (SFWO file:1-1-00-F-0162).

Giant Garter Snake (Thamnophis gigas)

Mosquito control activities in giant garter snake habitat may affect giant garter snakes by harassment or injury from vehicle use. The District will only operate vehicles in existing roads; therefore, harassment or injury from vehicle use would occur only if snakes are in the roadway. Regarding the effects of the proposed pesticides, a Fish and Wildlife Service-sponsored study indicated that the short-term effects of adulticides approved for mosquito control on the Sacramento NWRC did not significantly reduce abundance or biomass of the snake's prey items, macro-invertebrates and fish, in treated wetlands (Lawler et al. 1997). However, no information is available on the toxicity of the proposed pesticides directly to the giant garter snake. Without further information, it must be assumed that exposure of giant garter snakes to these chemicals could result in direct impacts, such as loss or sublethal effects to individual animals. Adverse effects to the giant garter snake from mosquito control activities will therefore be minimized by avoiding any wetland habitat suitable for giant garter while applying chemical treatments for control of mosquitoes. The application of adulticides by dispersal vehicles will be planned to fog downwind of and outside a buffer of 300 feet away from permanent emergent wetlands.

Valley Elderberry Longhorn Beetle (Desmocerus californicus dimorphus)

Adverse effects on the valley elderberry longhorn beetle are not likely since the main mosquito abatement period (June-September) does not coincide with the period of adult beetle emergence (late April through mid-May or early June). Also, the riparian corridors that house the valley elderberry longhorn beetle, generally do not require treatment with chemical control agents. If control measures are needed in these areas, some granular applications of Bti or Altocid (Methoprene) may be used during February or March when adult beetles are not present.

Vernal Pools

The growth regulator Methoprene (Altosid or A.L.L) can have deleterious effects on vernal pool shrimp by delaying the development of adult shrimp and thus the number of eggs laid before the pools dry up. Because of the effects of Methoprene on fairy shrimp and a lack of information on how long the agent remains in the soil, use of the larvicide methoprene within vernal pools or swales at any time, in either wet or dry conditions, is prohibited (USFWS 2001).

The majority of vernal pools and seasonal swales will be dry during the main pesticide application period (June-October). In general, naturally functioning vernal pool habitats are not significant mosquito-producing habitat and should not require chemical treatments for control of mosquito larvae. A study of vernal pools in Sacramento County suggested that when mosquito larvae were present in the pools, productivity was limited to a narrow time period just prior to drying in late spring. Therefore, vernal pools do not contribute at all to mosquito productivity in winter and early spring. In the event that the use of a larvicide does become necessary in the vicinity of vernal pools, Bti, which is relatively specific to mosquitoes and flies, will be the agent of choice.

The majority of the vernal pools at the Refuge occur on the Wetland Preserve property which became part of the Refuge under a conservation easement in 2004. During the spring of 2004, before the conservation easement went into effect, numerous vernal pools were treated with Bti. Relatively warm spring temperatures in 2004 likely contributed to elevated larval populations, but other factors may also be involved. The mosquito abatement district had increased larval monitoring in the area because the Wetland Preserve property is adjacent to a new housing development and WNV had recently arrived in Sacramento County. Many of the vernal pools in the Wetland Preserve property are man-made mitigation pools that may not be functioning as naturally occurring vernal pools would. A study of naturally occurring and constructed vernal pools conducted by the District showed that while natural vernal pools produced very few mosquitoes throughout most of the wet season and then produced a spike in numbers in late April, the constructed vernal pools produced significantly more mosquitoes throughout the wet season as well as a spike in numbers in April (Wright 1997). In addition, the data suggested that natural vernal pools may pose a greater threat of mosquito productivity when associated with constructed pools. For these reasons, the mosquito abatement district policy is to dip-sample constructed vernal pools and adjacent natural vernal pools. Mosquito abatement treatments near vernal pools will be limited to Bti to reduce effects on endangered vernal pool species. Future mosquito abatement activities in the Wetland Preserve property will be closely monitored by Refuge staff to avoid conflicts between wildlife habitat improvement goals and mosquito control goals.

Wetlands and Waterfowl

The Refuge was established to provide habitat for migratory birds, in particular waterfowl. The District will continue to minimize disturbance and non-target effects to wildlife by limiting mosquito abatement activities between October 15 and February 15 when the majority of migratory bird species arrive on the Refuge. However, since the District continues to treat in fall until temperatures have dropped sufficiently to reduce the abundance of mosquitoes, in warmer years there may well be a longer period of overlap between the arrival of migratory birds and continued mosquito abatement activities. In addition, if mosquito thresholds are exceeded, or the presence of WNV or other arboviruses are detected in or around the Refuge, then the District may need to extend mosquito surveillance and control into late fall.

In some years, most notably 2004, the District has applied Bti or planted mosquito fish as early as March when some migratory waterfowl may still be lingering before departing on their spring migration. However, Bti has not been found to be toxic to birds (USFWS 2001). In addition, it has been found that birds are not negatively affected by utilizing foods exposed to Bti or methoprene (Niemi et al. 1999). Although physico-chemico data and environmental fate data are limiting, *Bacillus* spp. are virtually non-toxic to mammals, birds and fish. Though methoprene has not been shown to pose a threat to birds from direct exposure, it may affect insectivorous species by decreasing the invertebrate food source. However, during the last 8 years methoprene has not been applied prior to June, and was applied as late as October in only one instance. Thus, applications of methoprene have not directly or indirectly affected migratory birds utilizing the Refuge because migratory birds have not been present during mosquito abatement activities.

There is not likely to be much impact on geese and swans from pesticides because they are year round herbivores. Geese feed mainly on grasses and agricultural lands, while swans feed mainly on roots, tubers, stems, and leaves of submerged and emergent aquatic vegetation. In contrast, ducks are known to be opportunistic feeders on both plants and invertebrates, utilizing the most readily available food sources. Invertebrates, plants, and seeds compose the majority of their diet, varying with the season and the geographic location. A study in California's Sacramento Valley has shown that plant foods are dominant in fall diets of northern pintails, while invertebrate use increases in February and March (Miller 1987). Seeds of swamp timothy comprise the most important duck food in the summer-dry habitats of the San Joaquin Valley (Miller 1987). Waterfowl in general tend to feed on seeds when they reach their wintering areas, perhaps to regain energy lost during long flights (Heitmeyer 1988, Miller 1987). Thus any food chain impacts resulting from larvicide and adulticide treatments will have limited impacts to the mainly seed diet of newly arriving ducks. Their diet shifts to invertebrates after mosquito treatments are expected to be reduced in frequency, thereby allowing the invertebrate populations to recover.

Birds utilizing the Refuge during the summer months and early fall, when most of the mosquito abatement occurs, could have a greater risk of being affected by pesticide applications. These species include herons, egrets, white pelicans, mallards and wood ducks. The pesticides being applied at the Refuge have not been shown to be toxic to birds, but could potentially affect resident waterfowl indirectly by reducing invertebrate food sources. Shorebirds could also be of concern, since they feed on a wide variety of invertebrates all year, feeding which intensifies at the onset of spring migration. However, documentation of indirect food-chain effects have not come to light. Hanowski et al. (1997) studied 19 different bird species after collecting data on wetlands 2 years before treatment and 3 years after treatment of both Bti and methoprene applications and found no negative effects. Jensen et al. (1999) found that no decreases were detected in the biomass or abundance of aquatic invertebrates in seasonal wetlands from ultra-low volume applications of pyrethrin, permethrin, or malathion.

VII. Health Threat Determination

For the purpose of allowing the use of certain pesticides or bio-rational pesticides to control mosquitoes, a mosquito-borne public health emergency is defined as:

Actual or threatened, imminent outbreak of western equine encephalitis, St. Louis encephalitis, West Nile encephalitis, malaria, or other mosquito vector-borne public health disease. The presence of WEE, SLE, WNV or malaria viral titers or mosquito pool titers in the mosquito population or in sentinel chickens (in accordance with test protocols developed by the CDHS Environmental Management Branch, and the U.S. Department of Health and Human Services, Center for Disease Control) will confirm that a public health emergency exists or is imminent. This threshold will have been met when the mosquito abatement district notifies the Refuge manager of a laboratory test that is positive for any of the above viruses.

The recurring presence of arboviruses in the Central Valley since the 1940s has been well documented (Reeves 1987) such that the baseline health threat level at the Refuge is 2-3, depending on monitoring. Occurrences of WNV within Sacramento County in humans, domestic animals and wildlife are expected to increase in 2005 relative to 2004 (see Disease

History above) based on observed arboviral disease cycles. The health threat level for the Refuge is therefore 4-5 (see Table 4) for 2005 and may be elevated to 6-7 if an officially determined health emergency is declared due to WNV. Historically, the mosquito abatement response has been the same at threat levels 2-3 as in threat levels 4-5, that is, adulticides and pupacides have been approved for use by the Service based on the historical health threat rather than being reserved for use only when an existing health threat has been documented. As a result, mosquito larval control activities since 1994 have been largely limited to localized (less than five acres) applications of larvicides and until 2005, only three applications of adulticides.

Table 4. Example of Mosquito-Borne Disease Health Threat and Response Matrix

Current Conditions		Refuge Response	
Health Threat Category[1]	**Refuge Mosquito Populations** [2]		
No documented existing or historical health threat/ emergency	No action threshold	1	Remove/manage artificial mosquito breeding sites such as tires, tanks, or similar debris/containers.
Documented historical health threat/emergency	Below action threshold	2	Response as in threat level 1, plus: allow compatible monitoring and disease surveillance. Consider compatible nonpesticide management options to reduce mosquito production.
	Above action threshold	3	Response as in threat level 2, plus: allow site-specific compatible larviciding of infested areas as determined by monitoring.
Documented existing health threat (specify multiple levels, if necessary; e.g., disease found in wildlife, disease found in mosquitoes, etc.)	Below action threshold	4	Response as in threat level 2, plus: increase monitoring and disease surveillance.
	Above action threshold	5	Response as in threat levels 3 and 4, plus: allow compatible site-specific larviciding, pupaciding, or adulticiding of infested areas as determined by monitoring.
Officially determined existing health emergency	Below action threshold	6	Maximize monitoring and disease surveillance.
	Above action threshold	7	Response as in threat level 6, plus: allow site-specific larviciding, pupaciding, and adulticiding of infested areas as determined by monitoring.

1 Health threat/emergency as determined by Federal and/or State/local public health authorities with jurisdiction inclusive of Refuge boundaries and/or neighboring public health authorities.

2 Action thresholds represent mosquito population levels that may require intervention measures. Thresholds will be developed in collaboration with Federal and/or State/local public health authorities and vector control districts. They must be species and life stage specific.

VIII. Stipulations and Reporting

1. Every attempt will be made to minimize mosquito production through wetland design, habitat (water level and vegetation) management techniques, mosquito fish or other non-chemical treatments, before larvicides or adulticides are applied. Among chemical treatments, adulticides will be considered a last resort.

2. In keeping with the MOU, the Refuge will provide the District with an annual summary of planned Refuge water management and with notification of timings of flood ups and irrigations.

3. As required under the MOU, the District will provide the Refuge Manager with an annual operating plan for anticipated mosquito monitoring and control activities that may be needed on the Refuge during the upcoming year. The plan will provide for Refuge access requirements, control thresholds, and proposed larvicides and adulticides.

4. Mosquito control will be authorized on an annual basis by a Special Use Permit (SUP) issued by the Refuge. SUP conditions will reflect any applicable restrictions required under approved Pesticide Use Proposals or Section 7 Consultations.

5. The Refuge will submit to the CNO Office all required Pesticide Use Proposals to maximize likelihood of PUP approval prior to onset of upcoming mosquito season.

6. The District will notify the Refuge manager as soon as possible when mosquito larval thresholds (see IPM Plan, Figure 5) are exceeded and ground treatments are warranted.

7. When adult thresholds are exceeded, and in the event of a planned adulticiding, the District will contact and personally coordinate with the Refuge Manager or Assistant Refuge Manager prior to conducting the treatments to ensure control efforts do not conflict with routine Refuge operations.

8. The District will continue to consider environmental conditions, including water temperature, density of mosquito larvae, and presence of mosquito predators, when determining mosquitoes on the Refuge pose a threat to public safety and whether treatments are required.

9. To minimize pesticide drift, dispersing vehicles will follow routes on existing roads set up to fog downwind or outside buffers of 300 feet from areas supporting listed or proposed special status species, including vernal pools.

10. All chemical applications will occur when wind speeds are between 2 and 8 mph.

11. Any applications of mosquito adulticides will occur outside a buffer of 300 from any permanent emergent wetlands.

12. Application of mosquito control measures is to be conducted in accordance with approved Pesticide Use Proposals.

13. Mosquito control will be authorized on an annual basis by a Special Use Permit (SUP) issued by the Refuge. SUP conditions will reflect any applicable restrictions required under approved Pesticide Use Proposals or Section 7 Consultations.

14. At the end of the season and as required under the MOU, the District will provide the Refuge Manager with an annual report summarizing mosquito control activities during the previous year.

References

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Figure 5. Mosquito Monitoring and Control Protocol