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Effectiveness of Control Measures against Mosquitoes at a Constructed Wetland in Southern California

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ABSTRACT: The effectiveness of larvicide and adulticide treatments against mosquitoes at a constructed wetland in San Jacinto, California was assessed with larval surveys, trapping of emerging adults, and collections of host-seeking females by carbon dioxide-baited traps. *Bacillus thuringiensis* var. *israelensis* (*Bti,* Bactimos® pellets) applied at a rate of 19 kg/ha did not demonstrably affect *Culex* larval and emergent adult populations. Larval populations in the seven marshes of the wetland decreased from approximately one third-fourth instar larva/dip to undetectable levels following two applications of *Bacillus sphaericus* (Vectolex® CG) at a rate of either 19 or 23.6 kg/ha. The largest decline in the number of adult mosquitoes emerging per day from vegetated regions of the wetland occurred after *B. sphaericus* treatments. The *Culex erythrothorax* host-seeking population declined about 80-fold during September beginning three weeks after the first treatment with *B. sphaericus*; however, the *Culex tarsalis* host-seeking population did not decline abruptly until mid-October 1997. This result suggests that immigration of females from other developmental sites might be an important factor influencing the *Cx. tarsalis* host-seeking population at the wetlands. Safety concerns required that insecticide applications were carried out during daylight hours, and two daytime applications of adulticide (Pyrenone®) in early August were ineffective against mosquitoes resting in the thick vegetation.

Keyword Index: *Culex*, constructed wetlands, *Bacillus*, bacterial larvicides, bulrush.

INTRODUCTION

The incorporation of alternative water reclamation facilities, such as constructed wetlands, into water resource management programs is likely to affect vector control efforts. In order to fulfill the increasing demand for water and wastewater treatment in densely populated arid regions, such as southern California, alternative water management strategies will be needed. California has approximately 200 water reclamation facilities that recycle about 450,000 acre-feet annually (McCarthy 1997). The number of water reclamation facilities is projected to approximately double by 2000 (McCarthy 1997); most of the increase is projected to occur in southern California. Multipurpose, constructed wetlands are expected to play an important role in water reclamation programs. In addition to processing secondary- or tertiary-treated effluent, these wetlands provide habitat for wildlife and a site for public education on issues related to water and wildlife conservation. Rapid growth of vegetation in constructed wetlands quickly creates ideal conditions for mosquito development (Walton et al. 1996, Walton and Workman 1998). Mosquito production enhanced by constructed wetlands is likely to require increased mosquito abatement efforts, particularly where human populations continue to encroach on wetlands situated in previously agriculture-dominated regions.

In 1997, encephalitis virus activity in western Riverside and San Bernardino counties and large host-seeking adult mosquito populations at a multipurpose, constructed wetland in San Jacinto, California prompted the undertaking of control efforts against *Culex* populations. Seroconversion to SLE in sentinel chicken flocks in western San Bernardino and Riverside counties in early summer 1997 indicated a comparatively early onset of annual virus activity in the region (cf. Emmons et al. 1990, Kramer et al. 1996). Marked increases in the host-seeking and larval mosquito populations were also observed at the wetland during 1997. Although the predominant host-seeking mosquito, *Culex erythrothorax* Dyar, collected during the first two years (1995-1996) after flooding the wetland is thought not to play a significant role in St. Louis encephalitis (SLE) transmission in southern California (Reisen et al. 1992b). Large populations of host-seeking *Culex tarsalis* Coquillett were collected at the wetland during the summer (approximately 1,000 females per trap night in 1996: WEW, unpublished data). *Culex tarsalis* is the primary vector of SLE and western equine encephalomyelitis (WEE) to humans in rural areas of southern and central California (Reeves and Hammon 1962, Reeves 1990).

We report here on the efficacy of control efforts against mosquitoes associated with a 10 ha multipurpose, constructed wetland in southern California. Larval surveys, trapping of emerging adults, and collections of host-seeking females by carbon dioxide-baited traps were used to evaluate the effectiveness of larvicides and adulticides applied to the entire wetland.

SITE DESCRIPTION

The 10 ha demonstration wetland is located at Eastern Municipal Water District's Hemet-San Jacinto Regional Wastewater Reclamation Facility (HSJRWRF) in San Jacinto, California. The demonstration wetland consisted of five treatment wetlands (inlet marshes 1-5), a central pond, and two outlet wetlands (outlet marshes A and B) (USBR, NBS, and EMWD 1994, Walton et al. 1997). The wetland received approximately one million gallons daily of secondary effluent from the HSJRWRF.

The wetland was planted in autumn 1994 with two species of bulrush, *Schoenoplectus* {= *Scirpus*} *californicus* (Meyer) Soják and *S. acutus* (Muhl. ex. Bigel.) Löve and Löve. In 1997, the wetland was in the third year of operation and the surface area was approximately 70% covered by vegetation (Thullen et al. 1998). The inlet and outlet marshes contained bands of vegetation separated by open water. The water depth within the inlet and outlet marshes was maintained between approximately 0.5 and 0.6 m during the summer. Water temperatures at the middle of the water column of the open water regions in the inlet and outlet marshes ranged between 21.5°C and 26.5°C during the summer.

METHODS AND MATERIALS

Treatment of the wetland was carried out using a helicopter by a local pest control company under contract with the water district. Pelletized *Bacillus thuringiensis* var. *israelensis* (*Bti,* Bactimos® pellets) or granular *Bacillus sphaericus* (Vectolex® CG) formulations were applied using a hopper-spreader apparatus suspended below the helicopter. The two *Bti* treatments were made in conjunction with an adulticide (Pyrenone®; 6.0% pyrethrins, 60% piperonyl butoxide; application rate: 3 quarts/30 acres = 0.014 kg AI/ha) during mid-August. The adulticide was applied by cold aspiration through an hydraulic spray rig suspended below the helicopter. These treatments were followed by approximately biweekly applications of *B. sphaericus* until mid-October and a final application in early November (TABLE 1). Treatment rate for the bacterial larvicides was either 19 kg/ha or 23.6 kg/ha. Because of restricted after-hours access to the site and safety concerns, treatments were carried out during daylight hours between approximately

TABLE 1. Larvicide and adulticide applications to the 10 ha demonstration wetlands at the Hemet-San Jacinto Regional Water Reclamation Facility during 1997.

Date	Larvicide	Quantity (kg/ha)	Adulticide	Quantity (kg AI/ha)
August 13	Bactimos	19.0	Pyrenone	0.014
August 20	Bactimos	19.0	Pyrenone	0.014
August 29	Vectolex CG	19.0		
September 12	Vectolex CG	23.6		
September 26	Vectolex CG	19.0		
October 10	Vectolex CG	23.6		
November 3	Vectolex CG	19.0		

10:00 A.M. and noon.

In order to assess whether larvicide and adulticide treatments had a demonstrable effect on mosquito populations, larval abundance in dip samples, population trends for emerging adult mosquitoes, and host-seeking adult abundance in CO_2-baited traps were examined. Immature mosquitoes were sampled by dipping along three transects in each marsh of the wetland. The three transects were positioned in each marsh at successive open water-vegetation interfaces from either the inlet weir or the southernmost outlet weirs. Samples were taken biweekly at five equally spaced stations along each transect. At each station, three 400 ml dips were taken, combined in a concentrator cup (mesh opening: 200 μm), and preserved with 95% ethanol. Developmental stage and abundance of immature mosquitoes were determined at 25X to 50X magnification using a stereo dissecting microscope. Stage III and IV larvae were identified to species using Loomis (1959) and Bohart and Washino (1978).

The number of adults emerging from the wetlands was determined from collections using eighty-four, 0.25 m^2 emergence traps. Collapsible emergence traps were constructed by affixing fiberglass window screen to a hinged wooden frame. The gently sloping sides of the trap concentrated emerging insects into a wide-mouth (16 oz.) Mason jar fitted with a removable plastic funnel. Quadrats (mean number of *Schoenoplectus* shoots/m^2 ± SD: 265 ± 98) were established within the seven marshes of the wetland and the bulrush was cut just above the water surface on June 26-27. Emergence traps were placed into the wetlands on July 1. Collections were made weekly from July 8 through September 26, except for the period between July 15 and July 22 when no jars were placed on the traps. At collection, the jar was removed from the apex of the trap and the funnel in each jar was plugged with cotton. The jars were returned to the laboratory where the collections were killed by freezing and then enumerated under a stereo dissecting microscope. Because *Schoenoplectus* grows rapidly and could upset emergence traps, clipping of emergent shoots was carried out twice each week.

Host-seeking female mosquitoes at the San Jacinto wetlands complex were collected using three carbon dioxide-baited traps. Mosquitoes were collected weekly from May 2 through October 30 and biweekly from February through April and from November through December 1997. Traps were run overnight from approximately 15:00 until 08:00. Mosquitoes were identified to species (Bohart and Washino 1978) and counted using a stereo dissecting microscope at 12X magnification.

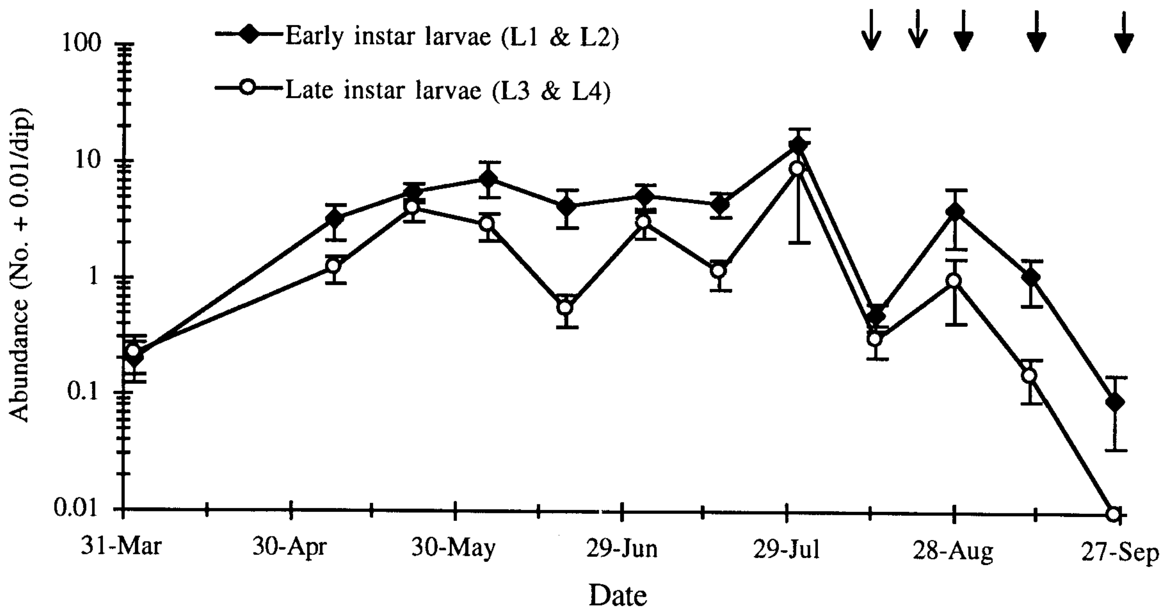
Effectiveness of the treatments against mosquito populations was indicated by a marked reduction in the abundance, or by an abrupt change in population growth, of a particular stage in the life cycle. The rate of population change (per day) for larvae and for emergent adults was calculated as: $\ln(N_{x+1}) - \ln(N_x)/t$, where N is the average number of larvae per dip or the average number of emergent adults/m^2/week, x is sample date, and t is the interval between samples in days. Larvae were divided into two subpopulations: early instar larvae (stages I and II) and late instar larvae (stages III and IV). The abundance of larvae and emergent adults was averaged for each of the seven marshes in the wetland by sample date. The predominant host-seeking mosquito, *Cx. erythrothorax*, found at the wetlands was typically under-represented in larval surveys (Walton and Workman 1998) and, therefore, the efficacy of control measures against this species was assessed using adults collected in emergence traps. Because the entire marsh was treated, both untreated areas and replicated experimental units were lacking.

Chemical and physical parameters related to water quality were routinely measured at the wetland. However, two parameters are particularly relevant to the results presented here: residual chlorine concentration and bacterial density. Residual chlorine in the water entering the wetland was measured using the iodometric method (APHA 1995). Coliform bacteria density in the influent water was measured using the fermentation tube test and calculated as the Most Probable Number (MPN) using the number of positive reactions in the dilution series (APHA 1995).

RESULTS

Larval Populations

Larval samples contained predominantly *Cx. tarsalis*. Late instar larvae of *Cx. quinquefasciatus* Say, *Cx. stigmatosoma* Dyar, and *Cx. erythrothorax* were less frequent in dip samples. The abundance of third and fourth instar larvae in dip samples increased from fewer than one larva per dip in April to an average of four larvae per dip in May (Fig. 1). The abundance of late instar larvae in the inlet marshes decreased in mid-June; in four of the five inlet marshes, larval abundance declined by nearly two orders of magnitude. Larval abundance in four of the inlet marshes on the next sampling date increased to levels observed in late May. For most dates during the summer, the average number of late instar larvae per marsh ranged between one and eight larvae per dip. In contrast to older larvae, the abundance of young larvae was comparatively stable from April through July, averaging between three and seven larvae per dip (Fig. 1).



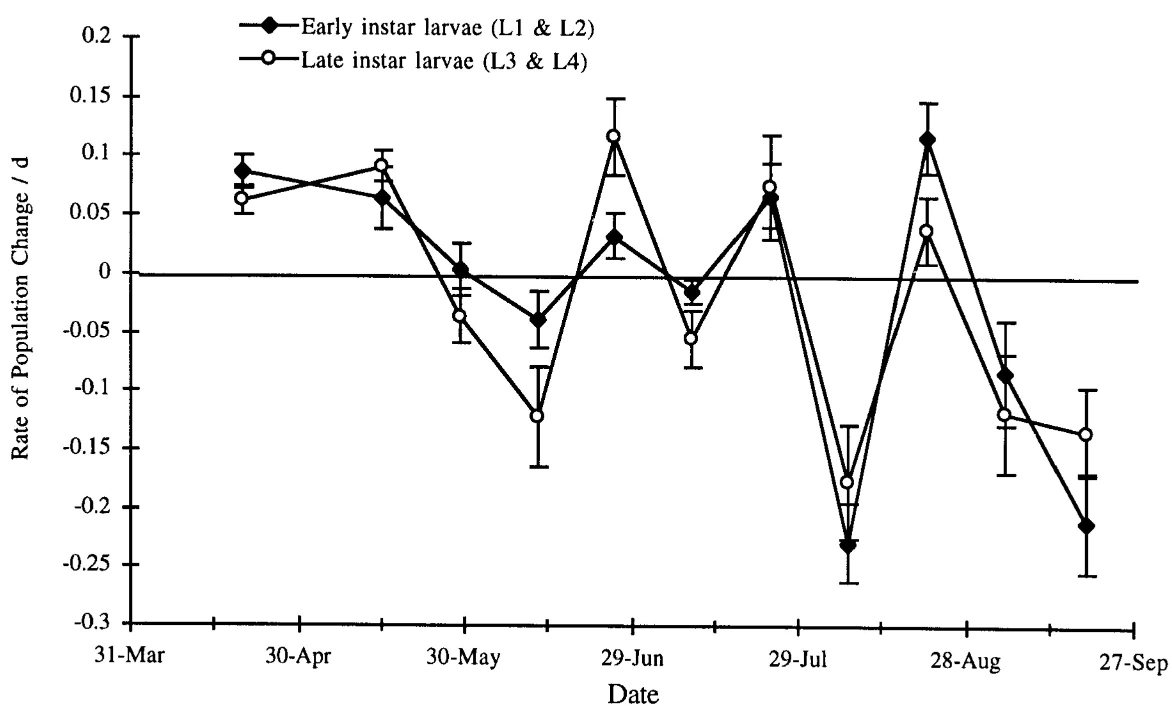


Figure 1. Population trends of *Culex* larval subpopulations in dip samples from the HSJRWRF demonstration wetland during 1997. Upper panel: abundance of larvae (mean ± SE) in dip samples. The arrows indicate treatments of bacterial insecticides. Open arrows are *Bacillus thuringiensis* var. *israelensis* treatments. Closed arrows are *Bacillus sphaericus* treatments. Lower panel: the rate of population change (mean ± SE) for two larval mosquito subpopulations.

The abundance of both larval subpopulations declined during August and late instar larvae were not collected from all marshes in late September (Fig. 1). In mid-August, larval abundance declined by more than ten-fold and averaged only 0.3 larva per dip. Larval counts in the inlet marshes were lower than in the outlet marshes at the end of August. By mid-September, the abundance of late instar larvae was below detectable levels in inlet marshes 2 and 5 and remained at this level in the last set of samples from late September. On September 26, late instar larvae were absent from all seven marshes.

Three periods of comparatively large negative population change (< - 0.1/d) were observed in the late instar larval subpopulation during 1997. These large decreases in growth rate of the late instar larval population occurred during early June (- 0.122/d), early August (- 0.176/d), and during September (< - 0.118/d: Fig. 2). The early instar larval subpopulation exhibited comparatively large declines in growth rate in early August (- 0.229/d) and during September (- 0.084/d, - 0.211/d). However, the negative growth rate of the older larvae observed in early June was not observed for the young larval subpopulation; the subpopulation of 1st and 2nd larval instars declined at 3%/d during the two week period in early June when the abundance of older instar larvae declined appreciably.

Adult Emergence

The number of *Culex* spp. emerging per square meter of vegetated surface declined gradually in all marshes between July 8 and August 15 (Fig. 2). On average, the number of mosquitoes emerging per unit area declined from approximately 1,500 adults/m^2/week in early July to 300 adults/m^2/week in early August.

The number of *Culex* emerging from the inlet marshes declined during the third week of August (Fig. 2). Mosquito production from the inlet marshes declined from 250 to 300 adults/m^2/week to 80 adults/m^2/week during the third week of August. Mosquito emergence from inlet marshes 1, 2, 4, and 5 increased during early September. Unlike the inlet marshes, adult production from the outlet marshes was nearly constant at 500 mosquitoes/m^2/week during August.

The most marked declines in adult mosquito emergence occurred after mid-September. Adult mosquito production decreased to about 25 mosquitoes/m^2/week between the second and third week of September. This was the largest negative change in population size (- 0.24/d), nearly twice the largest average decrease observed earlier in the summer (- 0.14/d: Fig 2).

The number of adult *Cx. erythrothorax* produced per quadrat was about ten times greater than *Cx. tarsalis* and more than one hundred times greater than *Cx. quinquefasciatus*. *Culex stigmatosoma* adults were rarely encountered in samples. *Culex erythrothorax* emergence declined approximately 10-fold between July 14 and July 28. Production for the second *Cx. erythrothorax* generation in 1997 was between 200 to 400 individuals/m^2/week from late July until early September, and then declined about 10-fold during September (Fig. 3).

The number of *Cx. tarsalis* females produced weekly from vegetated quadrats in the wetland declined at a rate of 7%/d during the summer (Fig. 3). An abrupt decline in adult emergence was not observed in early September; however, adult production was already very low at the end of August (< 10 females/m^2/week).

Host-Seeking Populations

In 1997, host-seeking populations of *Cx. erythrothorax* and *Cx. tarsalis* (Fig. 4) increased throughout April and May and reached annual maxima in June. The maximum number of host-seeking *Cx. erythrothorax* collected was nearly 33,000 individuals/trap night. The maximum number of host-seeking *Cx. tarsalis* collected was 4,560 individuals/trap night, about one-tenth of *Cx. erythrothorax*.

The *Cx. erythrothorax* host-seeking population declined approximately 80-fold during September (Fig. 4). Two periods of stasis in population numbers occurred in 1997 (Fig. 4). Catches of *Cx. erythrothorax* host-seeking females fluctuated around a mean abundance of 23,700 individuals/trap night from June 12 until the last week of August. After declining for a three-week period, the host-seeking population stabilized at an average of 475 females/trap night for four weeks (September 18 through October 16).

The *Cx. tarsalis* host-seeking population attained a maximum in June and declined slowly throughout the summer and early autumn (Fig. 4). The rate of decline for the host-seeking population in 1997 was 2.6%/d. Unlike the *Cx. erythrothorax* host-seeking population which declined abruptly in early September, *Cx. tarsalis* collections in carbon dioxide-baited traps fluctuated between 100 and 300 females/trap night during September and then declined abruptly in mid-October. Host-seeking females of both species disappeared in early November.

Water Quality

Residual chlorine concentration was above the limit of detection (0.2 mg/L) for the iodometric method on five dates between April and September 1997. Residual chlorine concentrations were greater than 1

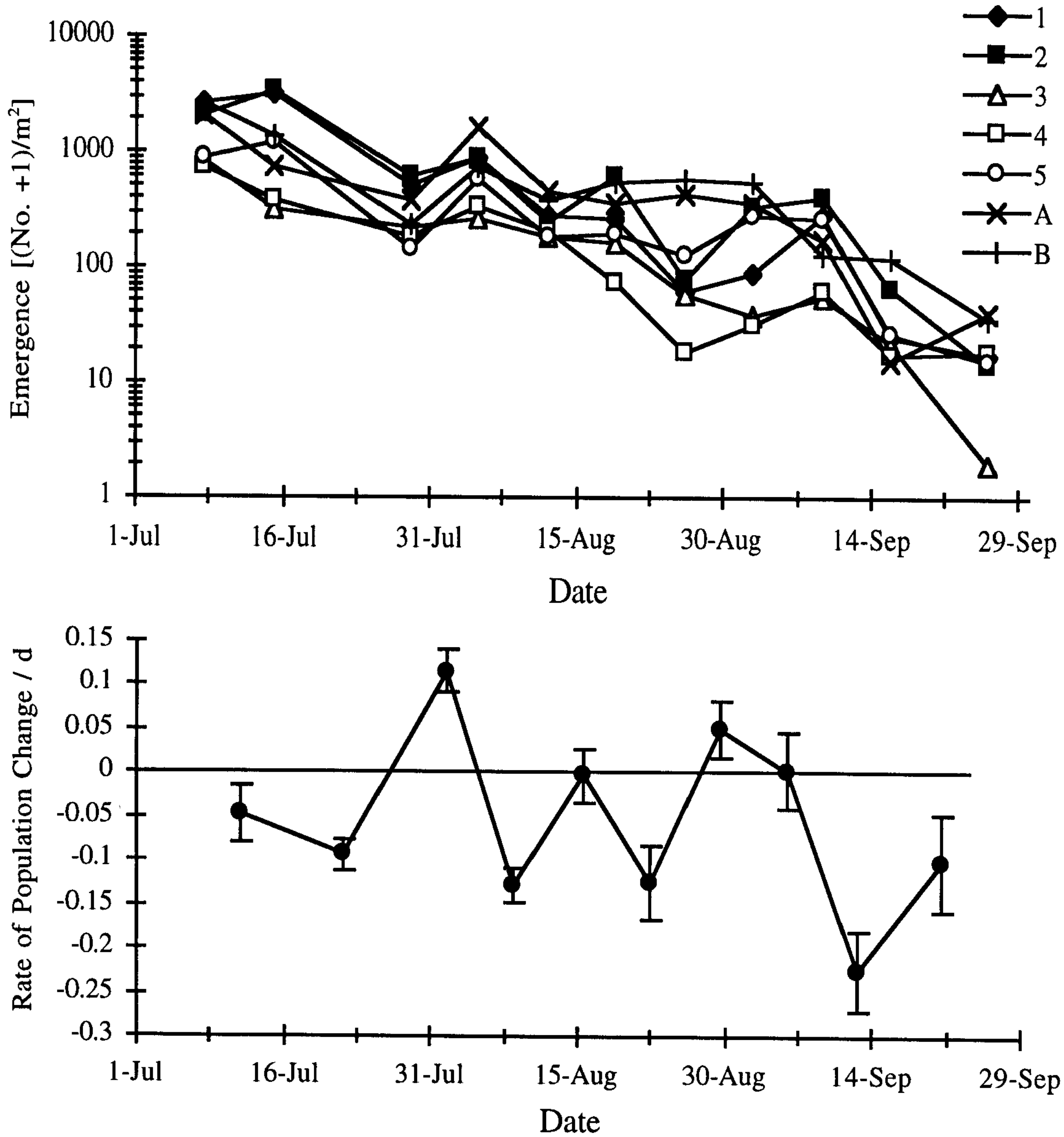


Figure 2. Number of *Culex* spp. emerging from the five inlet marshes (1-5) and two outlet marshes (A and B) in the HSJRWRF demonstration wetland during summer 1997. Upper panel: number of individuals emerging per week. Lower panel: the rate of population change (mean ± SE) for the emerging mosquitoes.

mg/L on three dates: the first week of May, a two week period in June-July, and at the end of August (Fig. 5). Increases in residual chlorine concentration were inversely related to bacterial abundance. Coliform bacteria densities declined by three or more orders of magnitude when residual chlorine concentration was greater than 1 mg/L (Fig. 5).

DISCUSSION

Wetlands constructed for wastewater treatment present a significant challenge for vector control agencies in the arid southwestern United States. The nutrients supplied by wastewater and the nearly year-round favorable growing conditions create dense stands of

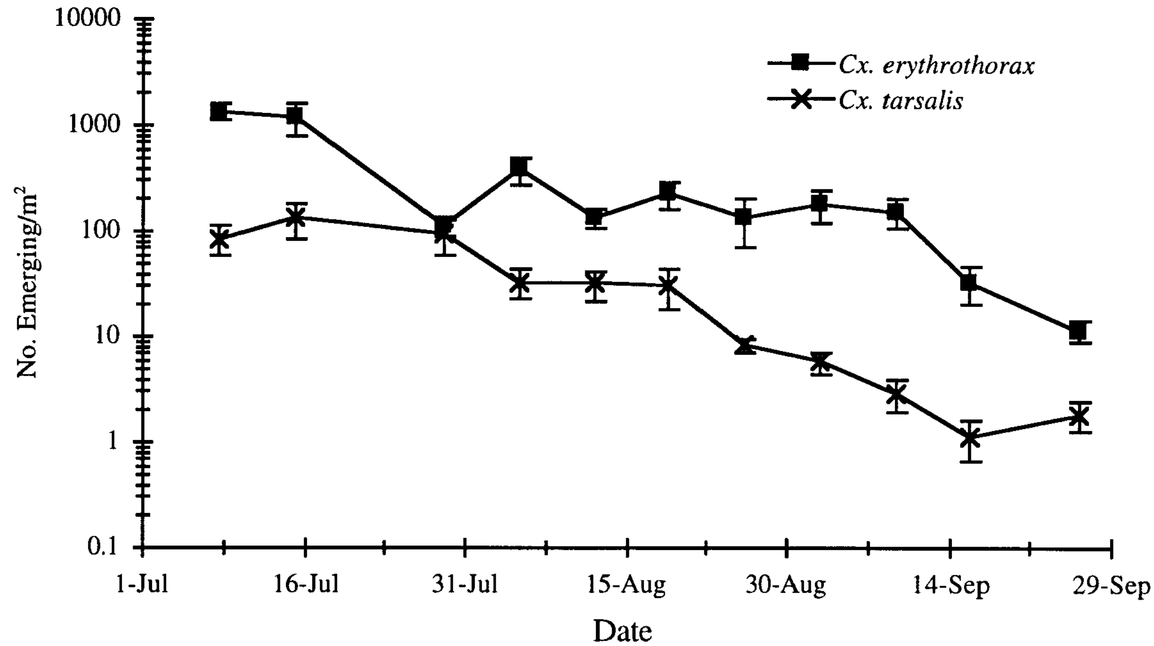


Figure 3. The average number (± SE) of *Culex erythrothorax* and *Culex tarsalis* females emerging from the seven marshes in the demonstration wetland, San Jacinto, California during 1997.

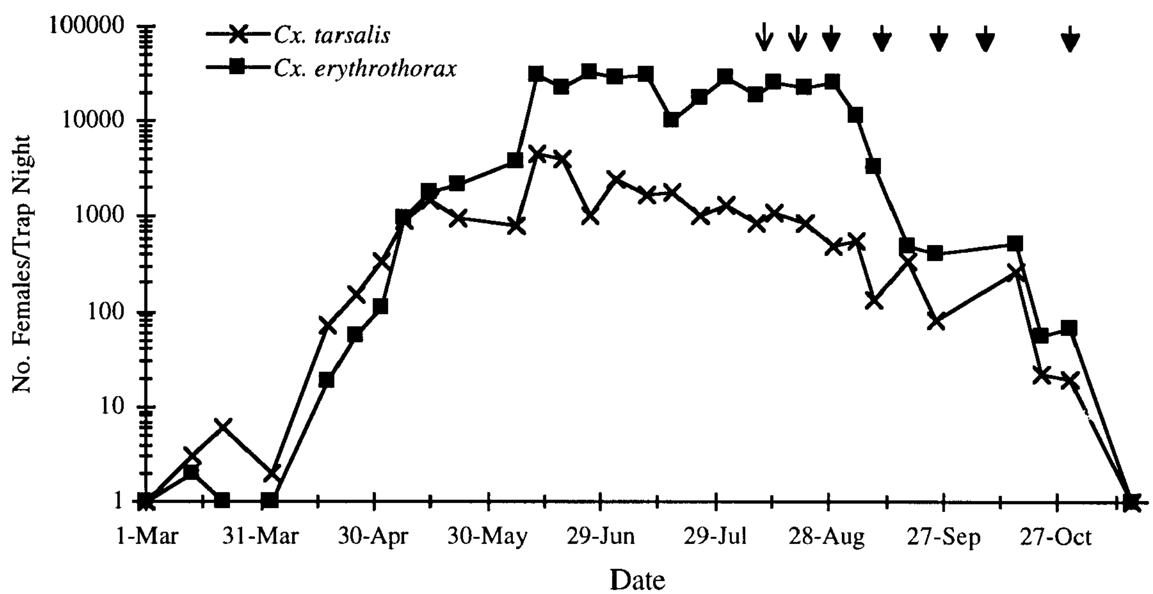


Figure 4. *Culex tarsalis* and *Culex erythrothorax* host-seeking populations collected by carbon dioxide-baited trapping at the demonstration wetland, San Jacinto, California during 1997. The arrows indicate treatments of bacterial insecticides. Open arrows are *Bacillus thuringiensis* var. *israelensis* treatments. Closed arrows are *Bacillus sphaericus* treatments.

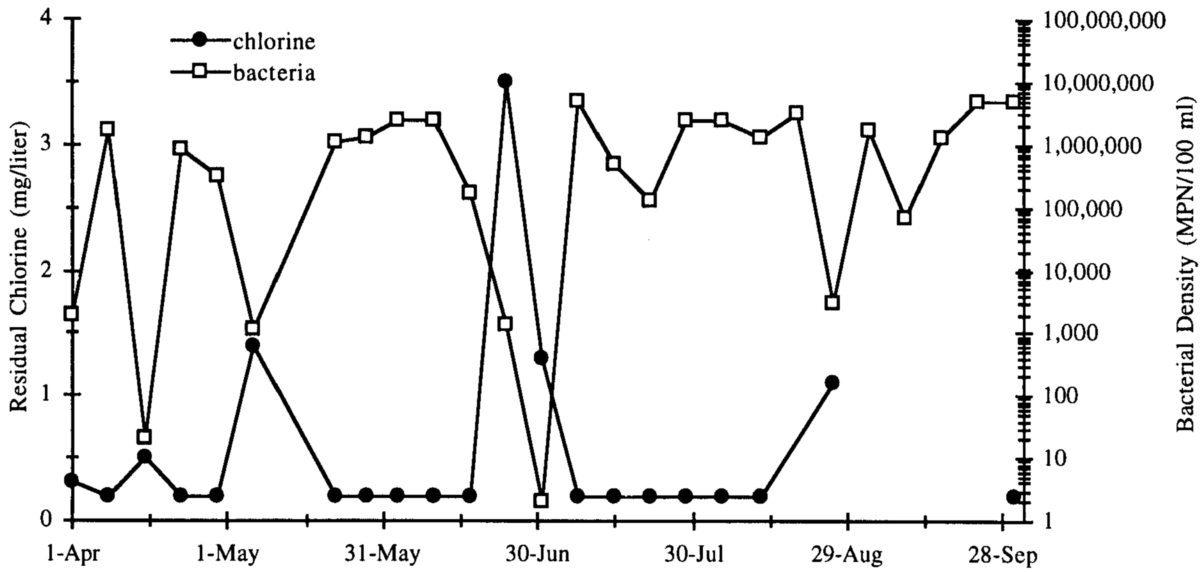


Figure 5. Residual chlorine concentration and coliform bacterial density in influent water to the HSJRWRF demonstration wetland, San Jacinto, CA during 1997.

wetland vegetation. Mosquito populations increased concomitantly (Walton, unpublished data) as vegetation densities increased and lateral growth of bulrush, particularly *S. californicus*, reduced the proportion of open water habitat at the San Jacinto wetland during the initial three years of operation (Thullen et al. 1998). In 1997, the adult host-seeking *Culex* spp. population averaged nearly 30,000 females/trap night during the summer. The large populations of resident and migratory birds that utilize constructed wetlands serve as potential reservoirs of arboviruses (Reeves 1990). Rapid human development in regions surrounding constructed wetlands can create an important public health concern especially when mosquitoes capable of vectoring disease, such as *Cx. tarsalis*, can readily move from developmental sites into human neighborhoods.

Bacterial larvicides provide an alternative to chemical insecticides or mosquitocidal oils for mosquito control at constructed wetlands where effluent water quality is an important consideration. However, thick stands of vegetation and other environmental factors limit the effectiveness of bacterial larvicides (Walton and Mulla 1992). Thick stands of bulrush limit the effective application of pelletized and granular formulations of the bacterial larvicides to constructed wetlands. The width of vegetated regions in the San Jacinto wetland (e.g., nearly 83 m in places) precludes the application of larvicides by backpack and truck-mounted application devices. Environmental factors such as temperature, salinity, and suspended solids influence the effectiveness and persistence of bacterial toxins (reviewed in Walton and Mulla 1992). For constructed wetlands receiving secondary-treated effluent, *Bti* is less effective in organically enriched water than is *B. sphaericus* (Mulla et al. 1990).

Bti did not have a demonstrable effect on larval and emergent adult populations at the San Jacinto wetland. Although larval and emergent adult populations declined appreciably in the inlet marshes after application of *Bti*, larval and emergent adult mosquito populations in the outlet marshes were not affected by the treatments. Because the entire wetland was treated with *Bti*, some other factor was presumably associated with the decline in the larval and emergent adult populations in the inlet marshes observed in August. The comparatively high levels of residual chlorine (> 1 mg/L) observed in the influent water during late August were directly related to marked declines in both the older larval subpopulation and the number of adults emerging from the inlet marshes. A similar concurrent decline of mosquito populations in the inlet marshes with high levels of residual chlorine in the influent water was observed in June.

The declines in larval abundance and larval population growth rate during September were associated with *B. sphaericus* treatments. Larval abundance declined by 80% in samples taken two weeks after treatment of the wetland with Vectolex. Two weeks later, third and fourth instar larvae were below detectable levels throughout the wetland. The greatest negative population change for mosquito larvae occurred after the *B. sphaericus* treatments. Even though residual chlorine was measured only once after August 29, high coliform bacterial abundance in the influent water during September indicated that residual chlorine did not cause the decline in mosquito populations. Larval surveys indicated that *B. sphaericus* was effective against the prevalent species in dipper samples, *Cx. tarsalis*.

Bacillus sphaericus was also effective against the most prevalent host-seeking mosquito in the wetland, *Cx. erythrothorax*. *Culex erythrothorax* larvae were under-represented in larval surveys from adjacent experimental wetlands (Walton and Workman 1998) and in dip samples from the demonstration wetland. Emergent adults provided the best indirect measure of the effectiveness of bacterial larvicides on the *Cx. erythrothorax* larval population. The largest declines in the emergent adult population followed the Vectolex treatments. Furthermore, the *Cx. erythrothorax* adult host-seeking population declined nearly two orders of magnitude after treatment of the wetland with *B. sphaericus*.

The lack of a marked decline in the *Cx. tarsalis* host-seeking population after treatment of the wetland with bacterial larvicides suggests that immigration of females from peripheral sources might be an important factor contributing to the maintenance of host-seeking populations at the wetlands. *Culex tarsalis* adult production from vegetated quadrats was already low (approximately 5 females/m^2/week) in late August, and even though the number of *Cx. tarsalis* adults emerging from the wetland declined after the larvicide treatments, the host-seeking population did not decline abruptly until October. *Culex tarsalis* adult female populations decline naturally during autumn. The timing of the decline for female abundance at the San Jacinto wetland was intermediate to the phenologies observed in New Jersey light trap samples from the cooler, more northern Central Valley and from the hotter, agricultural valleys in southern California (Reisen and Reeves 1990). Mark-recapture studies carried out during 1995 indicated that *Cx. tarsalis* host-seeking females could move two or more kilometers in one night and were also produced in large numbers at three sites surrounding the wetland (WEW, unpublished data). The maximum distances moved by marked female *Cx. tarsalis* have been estimated at between approximately 10 and 40 km (Bailey et al. 1965, Dow et al. 1965, Reisen et al. 1992a). In contrast to *Cx. tarsalis*, *Cx. erythrothorax* host-seeking females moved an average of 0.5 km per night, failed to disperse farther than 2 km from the wetland, and were concentrated only at the wetland and not at other developmental sites within a 3 km radius of our study site (Walton et al. 1998).

The difference in the dispersal tendencies of the two dominant *Culex* may have a very important consequence for control programs that utilize *B. sphaericus*. Unlike *Bti* which contains multiple toxins and does not readily promote the evolution of resistance in mosquitoes (Georghiou and Wirth 1997), *B. sphaericus* has a pair of mosquitocidal toxins (Baumann et al. 1991) which have been demonstrated in laboratory studies (Georghiou et al. 1992, Rodcharoen and Mulla 1994) to increase the risk for resistance. A recent study (Nielsen-LeRoux et al. 1997) indicated the involvement of multiple mechanisms in low-level (e.g., approximately 30-fold resistance after 30 or more generations of laboratory selection) versus rapid (< 8 generations) evolution of high levels (> 10,000-fold) of *B. sphaericus* resistance in the *Cx. pipiens* complex. Different levels of resistance in *Cx. quinquefasciatus* to *B. sphaericus* have also been demonstrated in several field studies (Sinègre et al. 1994, Rao et al. 1995, Silva-Filha et al. 1995). Because host-seeking *Cx. erythrothorax* females do not move very far from their natal developmental site, and if males exhibit similar dispersal tendencies, this mosquito has a greater potential to evolve resistance to *B. sphaericus* toxins than does *Cx. tarsalis*. Out-crossing of *Cx. erythrothorax* individuals from treated areas with susceptible individuals from untreated sites is less likely than for *Cx. tarsalis*. Because of their greater tendencies for dispersal, *Cx. tarsalis* populations from *Bacillus*-treated wetlands are likely to mix to a greater extent with susceptible populations from other developmental sites that either are untreated or where an alternative larval control is utilized. Cross resistance to other compounds used for mosquito abatement (e.g., *Bti*, methoprene, etc.) is unlikely (Nielsen-LeRoux et al. 1997).

Our analyses suggest that *B. sphaericus* was effective against *Culex* at this wetland; however, the effectiveness of the larvicide treatments should be interpreted cautiously. The timing of the declines for the larval populations, for emergent adult *Cx. erythrothorax*, and for host-seeking adult *Cx. erythrothorax* are suggestive that bacterial larvicides affected the mosquitoes. The results might be viewed as equivocal because the declines in the mosquito populations occurred towards the end of the season and there were no untreated populations against which to compare the effects of the larvicides. Clearly, replicated plots, untreated controls, and post-treatment samples closer to application dates (e.g., within 48 hours for *B.*

sphaericus treatments) would facilitate a less ambiguous statement of the efficacy of bacterial larvicides at the wetland. Biweekly larval surveys, intended for interannual comparisons of larval populations, might not detect a short-lived (i.e., < 2 weeks) reduction in larval abundance by the bacterial larvicides. Short-lived control of mosquito larvae by bacterial larvicides has been observed in other organically enriched habitats. *Bacillus sphaericus* reduced *Culex* larval populations in treated plots relative to untreated plots for one to three weeks in *Schoenoplectus* {=*Scirpus*} - *Typha* wetlands (Yoshimura et al. 1996) and in dairy wastewater lagoons (Mulla et al. 1988, Binding et al. 1996). *Bacillus sphaericus* was however effective for three weeks or longer against mosquitoes in catch basins (Siegel and Novak 1997) and in polluted water (Mulla et al. 1997). Yet, environmental factors (e.g., precipitation) had a marked effect on longevity of control in highly polluted mosquito developmental sites in Thailand (Mulla et al. 1997). *Bti* and *B. sphaericus* have proven only partially effective against *Culex* at treatment wetlands in Arizona because thick vegetation inhibited penetration of the larvicides (Levy 1997). Nevertheless, trends for the emergent adult populations at the San Jacinto wetland indicated that significant, short-lived reductions in larval abundance between sampling dates did not occur.

The projected increase in the use of constructed wetlands in regions of rapid human population growth is likely to cause a greater need for intensified vector control. A combination of integrated pest management strategies was needed to reduce both the larval populations and the large host-seeking populations at the San Jacinto wetland. The inability to effectively control the adult mosquito population, and continued recruitment into aquatic stages of the life cycle from the resident adult population, slowed mosquito abatement. In order to reduce mosquito populations, larviciding and effective adulticiding needed to be carried out concurrently. Daytime application of adulticides was ineffective; adulticiding would have been more effective during the two to three hours after dusk when the mosquito adults were most active.

The addition of larvivorous fish to the wetland and the establishment of an effective vegetation management program will greatly aid vector control efforts. Whereas, integrated control measures using mosquitofish and source reduction were effective in an urban wetland in southern California (Pelsue 1986), wetland managers have been reluctant to stock mosquitofish into constructed wetlands because of concerns related to clogging of pipelines moving reclaimed water or, after several months of effective mosquito control, mosquitofish were ineffective after the vegetation filled in wetlands (Levy 1997). There is a need to reevaluate wetlands operations procedures (e.g., changing access policies so that adulticiding can take place after dusk, establishment of effective vegetation management strategies) so that the public health and water needs of humans residing near man-made wetlands are fulfilled. Our results suggest that *B. sphaericus* may provide some measure of mosquito control in large-scale multipurpose constructed wetlands that receive organically enriched wastewater.

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