

# EFFECT OF MARSH DESIGN ON THE ABUNDANCE OF MOSQUITOES IN EXPERIMENTAL CONSTRUCTED WETLANDS IN SOUTHERN CALIFORNIA

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**ABSTRACT.** The species composition and abundance of larval mosquitoes were studied in the vegetated regions of 2 types of experimental constructed wetlands: one-phase marshes, which have continuous vegetation throughout the marsh, and 3-phase marshes, which have 2 vegetated regions separated by a region of comparatively deeper open water. Larvae of *Culex* spp. were significantly more abundant in one-phase marshes than in 3-phase marshes. Larval populations in one-phase marshes also contained proportionately more older larval instars (stages III and IV) than did populations in 3-phase marshes. Mortality rates of larvae increased during the summer and were higher in 3-phase marshes than in one-phase marshes during the initial 6 wk of the study. Differences in mortality rates between marsh types were related to predator abundance during the first 6 wk of the study and thereafter were not strongly associated with predator populations. An infusion of decaying bulrush (*Schoenoplectus californicus*) stimulated oviposition by gravid *Culex stigmatosoma* more than by gravid *Culex quinquefasciatus* and *Culex tarsalis*. *Culex erythrorhax* was the most abundant host-seeking species collected in CO<sub>2</sub>-baited traps; however, larvae were rarely collected during routine dip sampling and egg rafts were never collected in oviposition studies.

**KEY WORDS** Constructed wetlands, *Culex*, mosquitoes, invertebrate predation, bulrush, oviposition, organic infusions

## INTRODUCTION

Multipurpose constructed wetlands are likely to have an increasing role in water management programs in the southwestern United States. Innovative approaches to water and wildlife conservation strategies are needed for 2 reasons. First, there is an ever increasing demand for wastewater treatment by the burgeoning human populations in arid regions of the United States, such as southern California. Second, water districts and government agencies charged with the preservation of natural resources need to mitigate loss of wetlands caused by development. Multipurpose constructed wetlands can provide both a cost-effective supplement to standard wastewater treatment and other public benefits such as wildlife conservation and public education.

The Multipurpose Wetlands Research and Demonstration Project in San Jacinto, CA, is a multi-agency (Eastern Municipal Water District [EMWD], U.S. Geological Survey [USGS], and U.S. Bureau of Reclamation [USBR]) project that focuses on the development of design, construction, and operational criteria for large wetlands intended to process wastewater. In addition to establishing a large model wetland (ca. 10 ha), the project is evaluating the hydrology, nutrient removal capabilities, and other variables in a group of small (0.1-ha) experimental wetlands. The California bulrush (also bulrush, tule, *Schoenoplectus californicus* [Meyer] Soják) is used to remove nutrients, particularly nitrogen, from secondary treated water and to provide habitat for wildlife in the experimental wetlands. However, vegetation that strips wastewater of nutrients and provides cover for waterfowl also cre-

ates harborage for mosquitoes. An understanding of the potential mosquito production by constructed wetlands and effective mosquito abatement strategies must be developed in conjunction with the aforementioned design criteria, particularly if such wetlands are to be situated near residential areas. This point is particularly important in southern California, where rapid development may situate human residences well within the flight range of disease-transmitting mosquitoes, such as *Culex tarsalis* Coq., and of other species whose anthropophilic blood feeding is a nuisance.

The purposes of this research were to examine the abundance, species composition, and population ecology of larval mosquitoes in 2 types of experimental marshes; to examine the population trends of the adult host-seeking populations; and to assess whether decaying bulrush influences oviposition by mosquito species associated with the wetlands.

## MATERIALS AND METHODS

**Study site:** Our studies were carried out in 8 experimental marshes (research cells: 14 m by 69 m) that were divided into 2 groups based on the spatial distribution of *S. californicus*. One-phase marshes contained vegetation throughout the entire marsh except for a narrow zone (approximately 3 m) at the inflow and outflow (Fig. 1). Water depth was approximately 0.5 m. Three-phase marshes consisted of inflow and outflow marshes that were separated by an area of open water that was 1.2 m deeper than the inlet and outlet marshes. Water depth in the inlet and outlet marshes was approximately 0.5 m; 3-phase marshes also were devoid of emergent vegetation near the inlet and outlet weirs.

## One-phase marsh



## Three-phase marsh

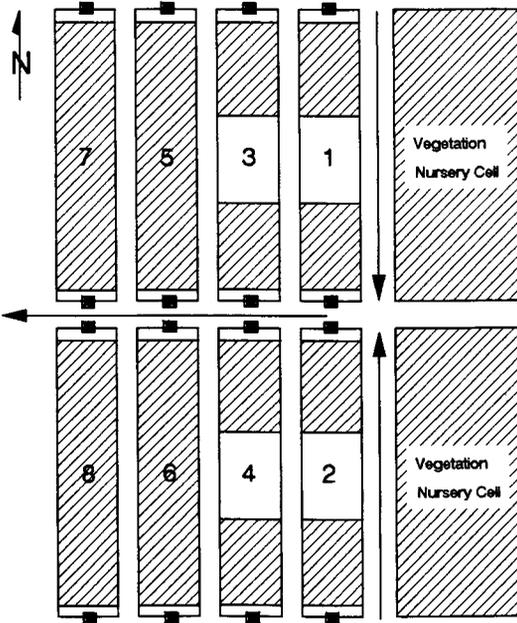
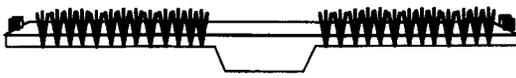


Fig 1. Schematic illustration of longitudinal cross sections and position of one-phase (cells 5-8) and 3-phase (cells 1-4) marshes at the Research Cell Complex, Eastern Municipal Water District, San Jacinto, CA. In the lower panel, cross-hatching represents vegetation and arrows indicate direction of water flow.

The experimental marshes were situated in 2 rows and received secondary-treated effluent from a single pipeline originating at the Hemet-San Jacinto Wastewater Treatment Facility in San Jacinto, CA. Two marshes of each type were located in each row (Fig. 1).

During 1995, flow rates in the experimental marshes were more variable (range: 0-228 liters/min) than during the first year of intensive water quality monitoring (1993 data: USBR, National Biological Survey [NBS] and EMWD 1994<sup>1</sup>); how-

ever, flow rates through the one-phase and 3-phase marshes averaged between 25.1 and 35.9 liters/min, respectively, in 1995 and were similar to those observed in 1993 (one-phase marshes: 23.1 liters/min, 3-phase marshes: 32.2 liters/min). Mean residence time of water was approximately 7 days (J. Thullen, personal communication). The temperature of incoming secondary-treated effluent from the treatment plant ranged between 24 and 27.5°C for the period May through November. Water temperature declined as effluent flowed through the marshes and the average temperature of the outlet water from the marshes was 7-8°C lower than that of the incoming water (25.5°C).

Natural turnover of emergent shoots and water-related stress contributed to a die-off of *S. californicus* in the research cells in the winter 1994-95. Consequently, the interior of the research cells contained dead material floating on the water surface during our study from June until September 1995. Healthy emergent stems were restricted primarily to the edges of the marshes. Bulrush had been planted into the experimental marshes in autumn 1992 and stem densities increased from <10 stems/m<sup>2</sup> in 1992 to 61-269 stems/m<sup>2</sup> (range of stem density calculated using data in USBR, NBS, and EMWD 1994<sup>1</sup>) in April 1993. By autumn 1993, the shallow marshes in the research cells were 100% covered by *S. californicus* and differences in bulrush abundance could not be discerned among the cells (USBR, NBS, and EMWD 1994<sup>1</sup>). Median stem density was estimated to have been approximately 330 stems/m<sup>2</sup>. The central ponds of the 3-phase marshes did not contain bulrush. On a per unit area basis, the 3-phase marshes contained approximately 67% of the vegetation present in the one-phase marshes.

**Larval mosquito populations:** Our sampling protocol assessed mosquito populations inhabiting a zone (1.5 m from shore) along the marsh periphery. We used this approach for several reasons. First, we were not permitted access to the interiors of a subset of the marshes because of ongoing hydrologic studies. Second, the interiors of the marshes were congested with downed bulrush and it was not possible to sample the water without disturbing larvae. In a subset of the marshes, mosquito larvae were sampled occasionally by dipping along transects cut through downed bulrush from the edge to the center of the marsh. Larvae were not concentrated along the transects and abundance of larvae in dip samples did not differ appreciably from the perimeter sampling described below; therefore, the samples taken along centrally oriented transects are not discussed further.

An initial survey of mosquito larval populations was conducted on June 26, 1995, by taking 5 400-ml dips at each of 32 stations along the periphery of each marsh. Mosquito larvae were absent along the periphery of the central ponds in the 3-phase marshes and sampling was discontinued in this re-

<sup>1</sup> U.S. Bureau of Reclamation, National Biological Survey, Eastern Municipal Water District. 1994. Multipurpose wetlands: phase II/III report. Final Design and Ongoing Research Investigations. September 1994. San Jacinto, CA.

gion so as not to confound comparisons with the vegetated habitats of the one-phase marshes. After the initial survey, the number of sampling stations per marsh was reduced to 16. The marshes were sampled weekly from July 7 until September 1. A final set of samples was taken on September 15. On each date, 3 400-ml dips were taken along a 2-m transect at each station. The samples were combined and preserved with ethanol. Because water levels in the marshes fluctuated and thick stands of hydrophilic terrestrial vegetation (i.e., willows) developed along the perimeters of some marshes, it was not always possible to take 3 dips at all stations as time progressed. Samples were returned to the laboratory and counted at 25 $\times$  under a dissecting microscope. Species identifications were made using larval stages III and IV (Loomis 1959, Bohart and Washino 1978). Statistical comparisons were based on the ln-transformed average number of larvae per dip for each research cell using a repeated measures analysis of variance (ANOVA).

Mortality rates for the young ( $v_i = 1$ st and 2nd instars) and old ( $v_{i+1} = 3$ rd and 4th instars) larval subpopulations were estimated using the vertical life table approach of Aksnes and Ohman (1996). The ratio of the numbers of individuals in the 2 subpopulations ( $r_i = v_i/v_{i+1}$ ) was used to estimate mortality ( $m$ ) as:

$$(r_i) = [\exp(ma_i) - 1]/[1 - \exp(-ma_{i+1})].$$

Stage durations ( $a$ ) in these food-rich, hypereutrophic environments are strongly influenced by temperature and were taken from *Cx. tarsalis* larvae reared in enclosures in the field (Walton, unpublished data) or were estimated from the laboratory studies of Bailey and Gieke (1968) and Mead and Conner (1987). Mortality estimates were obtained by iteratively solving the aforementioned equation.

**Adult mosquito populations:** Host-seeking females were collected weekly in a dry ice (CO<sub>2</sub>)-baited trap positioned at the center of the research cell complex. The trap was operated for one night each week from approximately 1500 until 0800 h from early July until December. During 2 mark-recapture studies, a trap was not run at the aforementioned position. The mean number of individuals collected in 4 traps positioned at the corners of the research cell complex was computed for September 15 and October 13.

**Macroinvertebrates:** The abundance of nonculline macroinvertebrates was determined weekly in dip samples and at 2-wk intervals (from July 14 to September 8) in minnow trap samples. A final set of minnow trap samples was taken on November 7. Gee minnow traps (Cuba Specialty Mfg. Co., Fillmore, NY) were lined with fiberglass window screen, baited with dog food, and placed in the corners of the marshes for 16–19 h. Minnow traps were deployed between 1400 and 1500 h and retrieved the following morning between 0730 and

0930 h. Minnow trap catches were processed in the field. The trap contents were placed into a white plastic pan and enumerated. Dip samples were preserved with ethanol, returned to the laboratory, and enumerated at 25 $\times$  under a dissecting microscope. The effect of marsh type on nonculline insects was tested by repeated-measures ANOVA using the ln-transformed abundance of taxonomic categories (family) and 2 functional (feeding) groups: predators of mosquitoes and herbivorous/detritivorous insects.

**Oviposition behavior:** The attractancies of marsh water or a tule infusion were examined in 3 oviposition studies. For experiment 1, 10 liters of water collected from the outflow of 2 marshes of each type (one-phase: cell 5 and cell 6; 3-phase: cell 2 and cell 3) were filtered through a 63- $\mu$ m mesh screen and placed in black plastic tubs (length  $\times$  width  $\times$  depth: 47 cm  $\times$  36 cm  $\times$  18 cm). Each treatment was replicated 4 times. Tubs containing water from each of the 4 research cells were separated by 3 m and placed adjacent to the 4 research cells. Oviposition was monitored for 2 consecutive nights (October 4–6). Oviposition data were analyzed statistically using a Kruskal–Wallis one-way ANOVA.

A tule infusion was used for experiments 2 (November 6–9) and 3 (November 14–17). Ninety grams of dead, dried *S. californicus* was pulverized with a blender and added to 20 liters of distilled water. The infusion was steeped for 1 wk at room temperature (22–23°C). This biomass of dried bulrush is equal to approximately 9.8 stems at 3.4 m in length; this amount of material suspended in 20 liters of water is equivalent to 245 stems/m<sup>2</sup>. This stem density is within the range of natural bulrush densities in the research cells.

On November 6, 4 replicates of 4 treatments were positioned as a Latin square experimental design in a field located to the north of the research cell complex. The 4 treatments were control (distilled water), full-strength infusion, and infusion diluted at 2 rates: 0.01 and 0.1 of full strength. Three liters of liquid was added to each tub and tubs were separated by 3 m. Oviposition by mosquitoes was monitored for 3 nights. On November 14, 4 treatments were blocked in 3 fields surrounding the research cell complex. The 4 treatments were control (distilled water), full-strength infusion (2 wk old), and infusion diluted at 2 rates: 0.33 and 0.67 of full strength. Three liters of solution was again placed into each tub and tubs were separated by 3 m. Oviposition was monitored for 3 nights. The square root transformation was applied to the quantity (number of egg rafts/tub + 0.5). The number of egg rafts deposited onto each treatment was compared by ANOVA and *a posteriori* comparisons among treatments were carried out using Tukey's honestly significant difference (HSD) test.

Egg rafts were returned daily to the laboratory, hatched individually, and larvae were reared to the

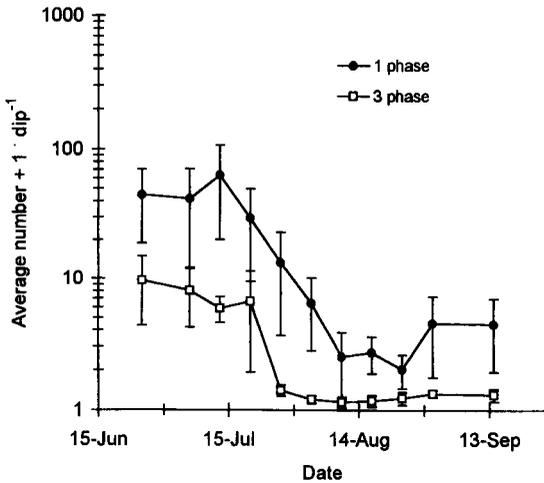


Fig 2. Abundance of *Culex* spp. larvae (mean  $\pm$  SD per dip) in vegetated habitats of one-phase and 3-phase marshes during 1995, San Jacinto, CA.

4th larval instar for identification during the 3 oviposition studies. During each of 3 oviposition studies, air temperature, wind speed, wind direction, and barometric pressure were measured hourly at a weather station located approximately 0.75 km from the research cell complex.

## RESULTS

### Mosquito Populations

Larval *Culex* populations in vegetated habitats of one-phase marshes were significantly larger than in the vegetation of 3-phase marshes (repeated-measures ANOVA:  $F_{1,6} = 8.12$ ,  $P < 0.028$ ). Numbers of larvae in dip samples differed, on average, between the marsh types by 5- to 10-fold during July and 2- to 3-fold thereafter (Fig. 2). Larval populations in 3-phase marshes declined markedly by the end of July and remained low through the end of the study in mid-September. Larval populations in one-phase marshes declined more slowly than in the 3-phase systems, reaching a minimum during mid-August. A slight resurgence in larval numbers was observed in 2 of the one-phase marshes during September.

The differences in larval populations between marsh types was attributable to differences in the abundance of early larval instars (stages I and II). The abundance of the young larval subpopulation in one-phase marshes was significantly greater than that in the 3-phase marshes (repeated-measures ANOVA:  $F_{1,6} = 8.54$ ,  $P < 0.027$ ). Greater numbers of the older larval subpopulation (stages III and IV) were found in the one-phase marshes than were present in the 3-phase marshes; however, the differences in population size across the entire study were not statistically significant (repeated-measures ANOVA:  $F_{1,6} = 5.02$ ,  $P < 0.066$ ). Even though the

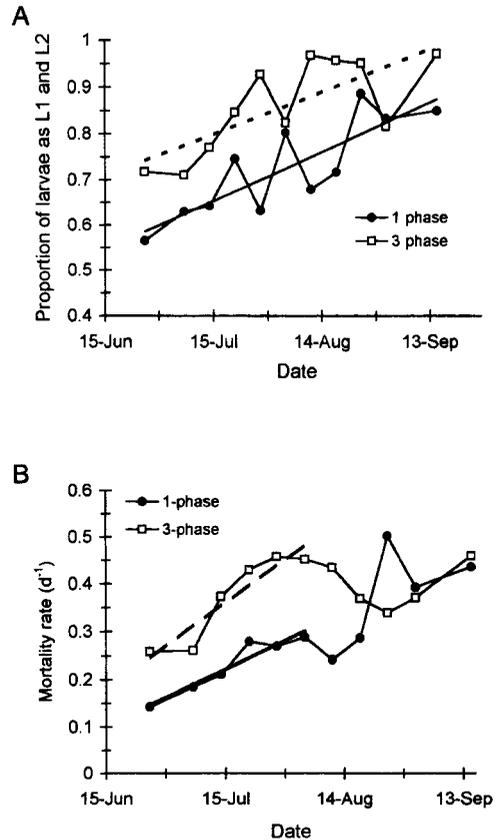


Fig 3. Proportion of the larval population composed of early instars (stages I and II) (A) and mortality estimates ( $m$ ) (B) for one-phase vs. 3-phase marshes. The best-fitting regression lines for the proportion of young larvae and mortality rate as functions of day of the year (Julian date) are illustrated for one-phase (—) and 3-phase (---) marshes. Proportion of young larvae ( $y$ ) vs. day of the year ( $x$ ): one-phase marshes:  $y = 0.0036x - 0.049$ ,  $R^2 = 0.71$ ; 3-phase marshes:  $y = 0.0030x + 0.206$ ,  $R^2 = 0.57$ . Mortality rate ( $y$ ) vs. day of the year ( $x$ ): one-phase marshes:  $y = 0.0035x - 0.468$ ,  $R^2 = 0.79$ ; 3-phase marshes:  $y = 0.0061x - 0.825$ ,  $R^2 = 0.87$ .

abundance of the older larval subpopulation did not differ statistically across the entire study, stage III and IV larvae were absent from 75% of the samples from 3-phase marshes taken between August 11 and September 15.

Larval populations in 3-phase marshes contained proportionately more younger instars than did the larval populations in the one-phase marshes (Fig. 3A). The proportion of young larvae (stages I and II) in dip samples differed significantly between the 2 marsh types (repeated-measures ANOVA using arcsine-transformed square root proportions:  $F_{1,6} = 13.29$ ,  $P < 0.011$ ). The fraction of the larval population composed of young larvae increased throughout the study from 56% in late June to approximately 83% in mid-September for the one-phase marshes and from 71% in late June to 85–

95% in mid-August through mid-September for the 3-phase marshes.

Mortality rates of larvae in 3-phase marshes were significantly greater than in the one-phase marshes during the initial 6 wk of the study (repeated-measures ANOVA:  $F_{1,6} = 16.48$ ,  $P < 0.007$ ). Mortality rates of larvae in one-phase marshes increased from approximately 0.13/day in June to 0.28/day in late July. During the period when larval populations were large, mortality rates of larvae in 3-phase marshes were comparatively higher (ca. 0.25–0.46/day) and increased faster (0.0061/day) than in one-phase marshes (Fig. 3B).

Mortality rates of larvae in 3-phase marshes during late August and September appeared not to differ substantially from that in one-phase marshes; however, larval mortality in 3-phase marshes was higher than is illustrated in Fig. 3B. Stage III and IV larvae were not present in most samples collected from 3-phase marshes after August 6 and, because the ratio of early to late instars was undefined (i.e., denominator equal to zero), mortality could be estimated for only one of the 3-phase marshes on each sampling date. Only these estimates are depicted in Fig. 3B.

In contrast to 3-phase marshes, older larvae were present in samples from one-phase marshes on all sampling dates. The mortality rate of larvae in one-phase marshes increased throughout the study (0.0038/day) and the increase in mortality rate per unit time did not differ significantly from that during the first 6 wk of the study (0.0035/day; comparison of slopes,  $t_{13} = 0.174$ ,  $P > 0.05$ ).

Mortality rates of *Culex* larvae were significantly associated with the abundance of predators and increased directly with predator abundance (ln-transformed) irrespective of marsh type (analysis of covariance [ANCOVA]: homogeneity of slopes:  $F_{1,44} = 0.006$ ,  $P > 0.93$ ; marsh type after controlling for predator abundance:  $F_{1,45} = 3.33$ ,  $P > 0.07$ ; predator abundance:  $F_{1,45} = 40.57$ ,  $P < 0.001$ ) from the last week of June through the first week of August (Fig. 4). In 3-phase marshes, the number of predators in dip samples was directly related to larval mortality during the first 6 wk ( $r = 0.75$ ;  $t_{22} = 2.33$ ,  $P < 0.05$ ). Larval mortality and predator abundance in one-phase marshes were less strongly correlated for the same period ( $r = 0.61$ ;  $t_{22} = 2.24$ ,  $P < 0.05$ ). The strength of the association between larval mortality and predator abundance declined during August and September (one-phase marshes:  $r = 0.20$ ; 3-phase marshes:  $r = 0.19$ ;  $t$ -tests,  $P > 0.05$ ).

*Culex tarsalis* predominated among the species collected as larvae during this study, making up more than 50% of the larvae collected on most dates (Fig. 5). Two other *Culex* species commonly associated with wastewater, *Culex stigmatosoma* Dyar and *Culex quinquefasciatus* Say, composed 20–40% and 5–20%, respectively, of the larvae collected. Three other species were rarely collected as larvae (<5% of the total larvae collected on

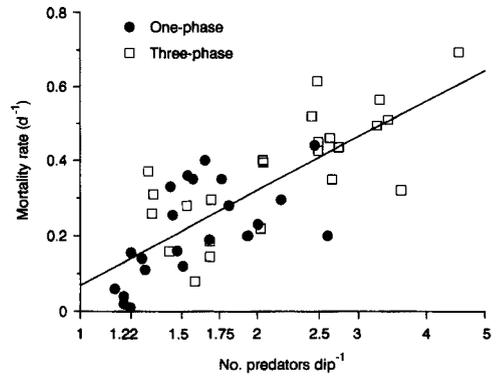


Fig. 4. Mortality rate of *Culex* larvae as a function of predator abundance in dip samples from 2 types of experimental wetlands during June 26 through August 6, 1997. The best fitting regression line for larval mosquito mortality rate ( $y$ ) versus predator abundance ( $x$ ) is shown:  $y = 0.365x - 0.063$ ,  $R^2 = 0.60$ .

most dates): *Culex erythrothorax* Dyar, *Culiseta inornata* (Williston), and *Anopheles franciscanus* McCracken.

Although these species were also collected as adults, their relative abundance in the host-seeking female population differed appreciably from that in larval surveys. *Culex erythrothorax* was the predominant species collected by  $\text{CO}_2$ -baited traps during the entire study (Fig. 6). Four cohorts of host-seeking females were observed between July and December. The maximum number of individuals caught for each cohort ranged between 2,000 and 2,500 individuals/trap/night from August until late October, and nearly 4,600 individuals/trap/night in November. *Culex tarsalis* was common during the late summer and much of the autumn, but was approximately 10 times lower in abundance than was *Cx. erythrothorax*. *Culex tarsalis* host-seeking populations declined during October. *Culex quinquefasciatus* was uncommon; fewer than 10 individuals were collected on most nights. Rarely encountered species included *Cs. inornata*, *An. franciscanus*, *Aedes dorsalis* (Meigen), and *Cx. stigmatosoma*.

### Macroinvertebrates

Predatory insects (e.g., notonectids, odonates, coleopteran larvae) in dip samples from 3-phase marshes were significantly more abundant during the first 6 wk of the study (repeated-measures ANOVA:  $F_{1,6} = 20.90$ ,  $P < 0.004$ ) than they were in samples from one-phase marshes (Fig. 7A). Notonectid abundance in 3-phase marshes was significantly greater than in the one-phase cells (repeated-measures ANOVA:  $F_{1,6} = 12.30$ ,  $P < 0.013$ ). Thereafter, predator populations fluctuated in size and no population trend was consistent among marshes of a particular type across the entire study (repeated-measures ANOVA:  $F_{1,6} = 2.66$ ,  $P > 0.15$ ).

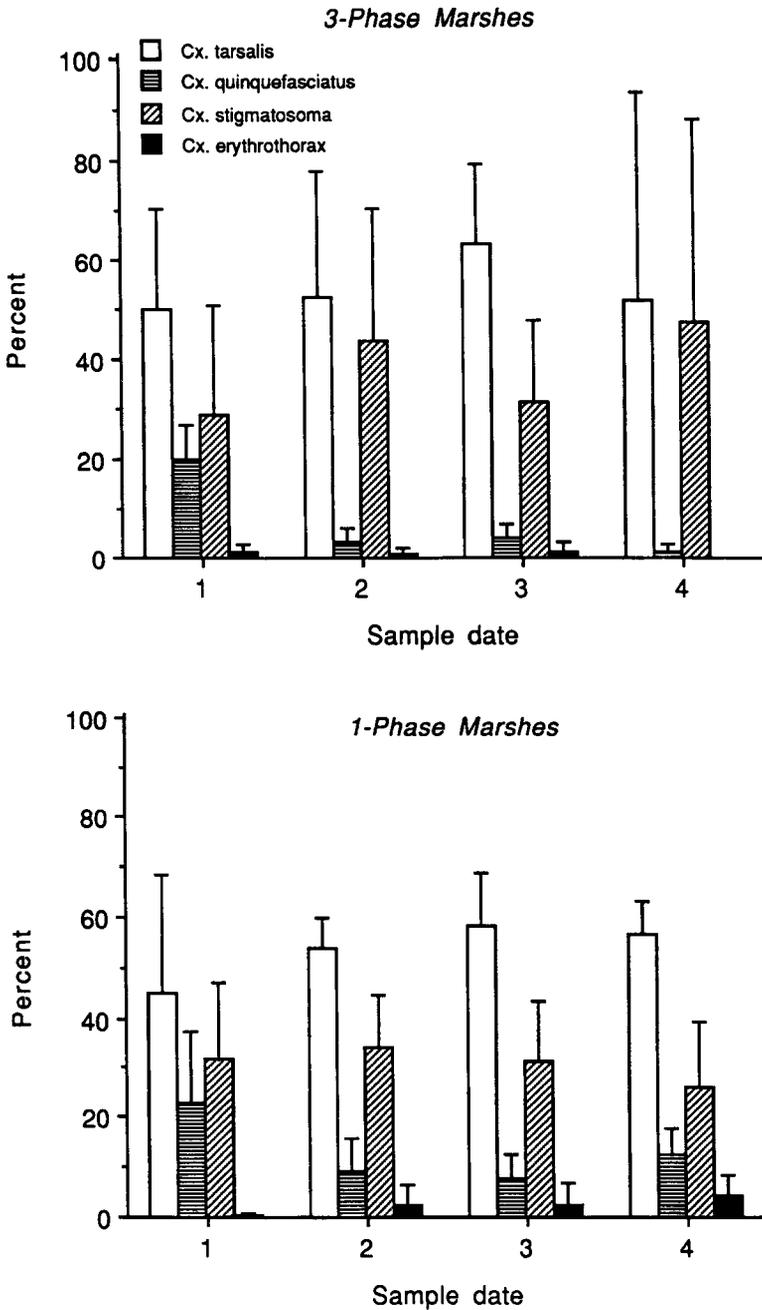


Fig. 5. Relative abundance of 4 *Culex* species in dip samples from 2 types of experimental marshes during June through July 1995, San Jacinto, CA. Error bars are 1 SE.

In contrast to the predatory insects, a nonsignificant trend (repeated-measures ANOVA:  $F_{1,6} = 2.18$ ,  $P > 0.19$ ) occurred toward greater abundance of herbivorous and detritivorous insects in dip samples from one-phase marshes than in 3-phase marshes during the first month of study (Fig. 7A). This functional group declined slightly in

abundance during August and then increased approximately 2-fold by mid-September in both marsh types. Across the entire study, marsh design did not significantly affect the abundance of the herbivore and detritivore functional group (repeated-measures ANOVA:  $F_{1,6} = 2.03$ ,  $P > 0.20$ ) or that of any family of herbivorous and detriti-

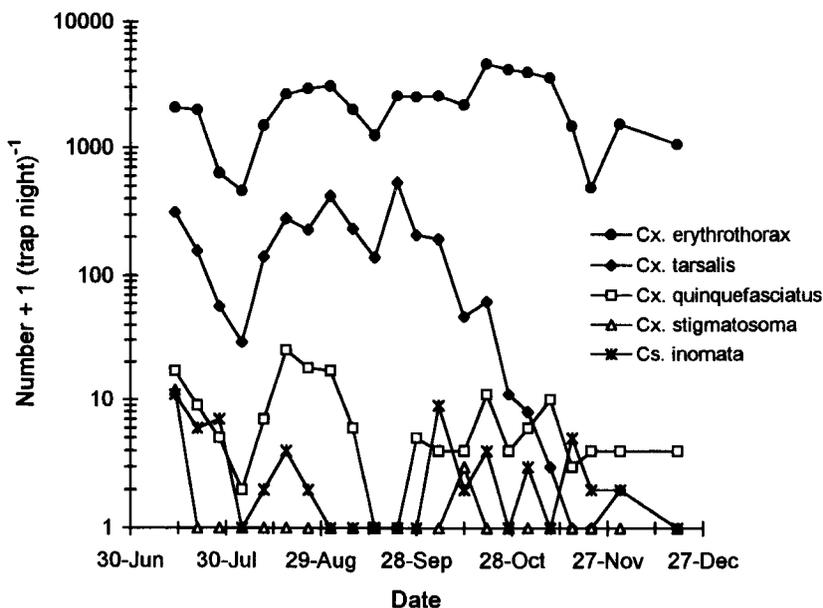


Fig 6. Abundance of host-seeking females per  $\text{CO}_2$ -baited trap at the Research Cell Complex, Eastern Municipal Water District, San Jacinto, CA, during 1995.

vorous insects (e.g., hydrophilid adults, chironomid larvae, corixids, baetid nymphs; repeated-measures ANOVAs by taxon:  $P > 0.05$ ).

Predators showed slight declines in the minnow trap catches across the study, whereas minnow trap catches of herbivores and detritivores declined by a factor of 10 from late June through early November (Fig. 7B). Marsh type did not significantly affect the abundance of either functional group throughout the entire study (repeated-measures ANOVAs,  $P > 0.05$ ). Although some overlap occurred in the organisms sampled by dipping and by minnow traps, minnow traps were more effective for sampling adult coleopterans (*Cybister explanatus* LeConte, *Hydrophilus triangularis* Say, *Rhantus anisonychus* (Aubé), *Rhantus gutticollis* (Say), *Thermonectes basillaris* (Harris), and *Tropisternus* spp.), larvae of large coleopterans, aeschnid (*Anax junius* (Drury) and *Aeschna multicolor* Hagen) nymphs, and belostomatids (*Belostoma flumineum* Say) than was dipping. Omnivorous corixids, which concentrate their feeding on the substrate and therefore are unlikely to consume mosquito larvae, primarily *Corisella decolor* Uhler and *Corisella inscripta* (Say), were the largest component of the herbivore and detritivore functional group.

### Oviposition

In experiment 1, mosquitoes oviposited preferentially onto water from research cell 6. Oviposition differed significantly among the marsh water from the 4 research cells (Kruskal-Wallis test:  $H_3 = 9.37$ ,  $P < 0.025$ ). Only 10 *Cx. stigmatosoma* egg

rafts were collected during this experiment and 80% of the egg rafts were deposited on water from cell 6.

The number of egg rafts deposited onto the tule infusion treatments differed significantly in experiments 2 and 3. For both experiments, significantly more egg rafts were collected from the full-strength treatment than were collected from the 2 dilution treatments and the controls. In experiment 2, 84% of egg rafts (total = 57) were collected from the full-strength infusion (Fig. 8). Even though experiment 2 was designed to account for possible environmental effects of wind patterns and topography, the effects of prevailing wind direction and distance from the road berm (row and column effects) were not significant ( $F_{3,38}$ ,  $P > 0.18$ ). The effect of dilution was highly significant ( $F_{3,38} = 29.01$ ,  $P < 0.001$ ). The number of egg rafts deposited onto the full-strength infusion differed significantly from the distilled water control and the 2 (0.1 and 0.01 of full strength) dilutions (Tukey's HSD:  $P \leq 0.05$ ). The infusion remained attractive/stimulatory for the duration of the experiment (3 days).

In experiment 3, two thirds of the egg rafts (total = 78) were deposited onto the full-strength infusion and approximately one fifth (22%) of the egg rafts were collected from 67% dilution treatment (Fig. 9). Significant differences in mosquito oviposition among treatments ( $F_{3,24} = 6.01$ ,  $P < 0.004$ ) were evident for the first 2 nights of the study. Mosquito oviposition onto the treatments differed significantly: full strength > 67% dilution > 33% dilution = control (Tukey's HSD:  $P \leq 0.05$ ). The effects of

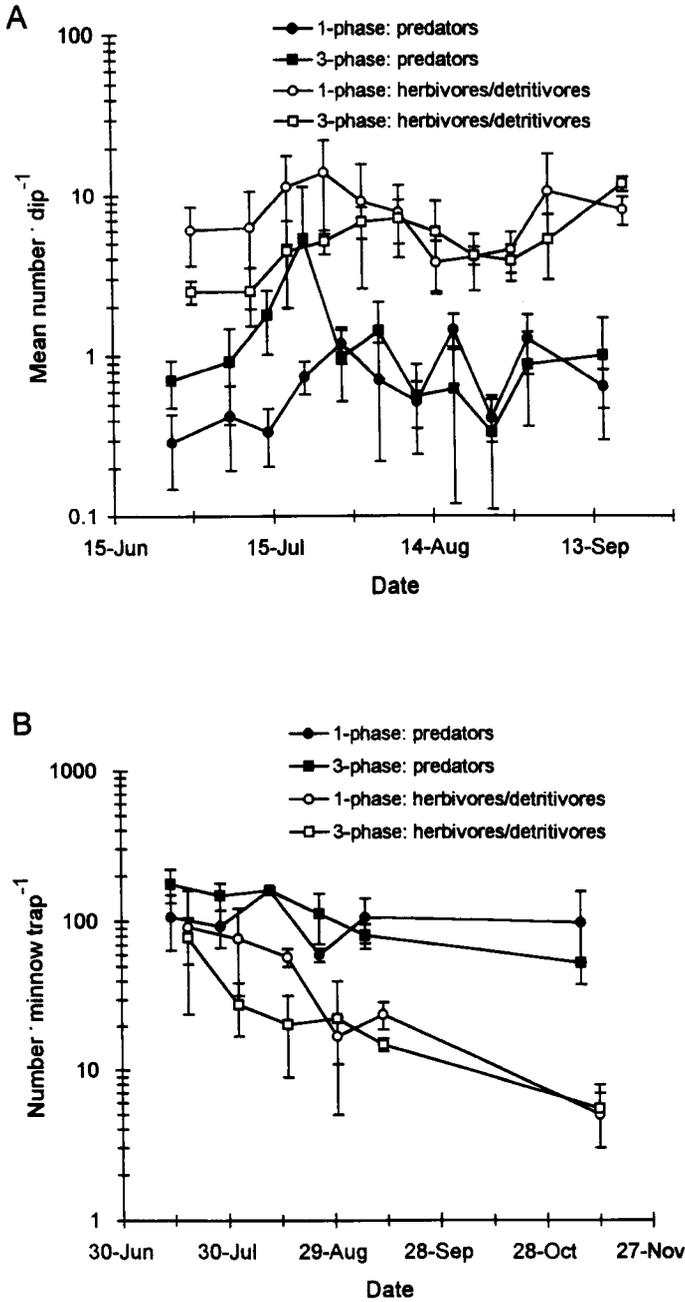


Fig 7. Macroinvertebrate predator and noncucicine herbivore and detritivore abundance in dip samples (A) and minnow traps (B) from 2 types of experimental marshes in San Jacinto, CA, 1995. Lines are offset horizontally to facilitate illustration. Error bars are 1 SD.

location (block:  $F_{2,24} = 0.48, P > 0.62$ ; block-treatment interaction:  $F_{6,24} = 0.72, P = 0.64$ ) were not significant. The intensity of oviposition declined abruptly on day 3 in experiment 3. Strong winds (7–9 mph) during the 4 h immediately after sunset presumably deterred oviposition on this date.

Mosquitoes whose larvae are typically found in foul water predominated among the egg rafts col-

lected from the tubs (Figs. 8 and 9). *Culiseta inornata* egg rafts were rare and were only found on comparatively clean water. The percentage of egg rafts deposited by species in experiment 2 was *Cx. stigmatosoma*, 77%; *Cx. quinquefasciatus*, 14%; *Cx. tarsalis*, 7%; and *Culiseta inornata*, 2% (Fig. 8). In experiment 3, the relative abundance of egg rafts collected from the oviposition tubs was *Cx.*

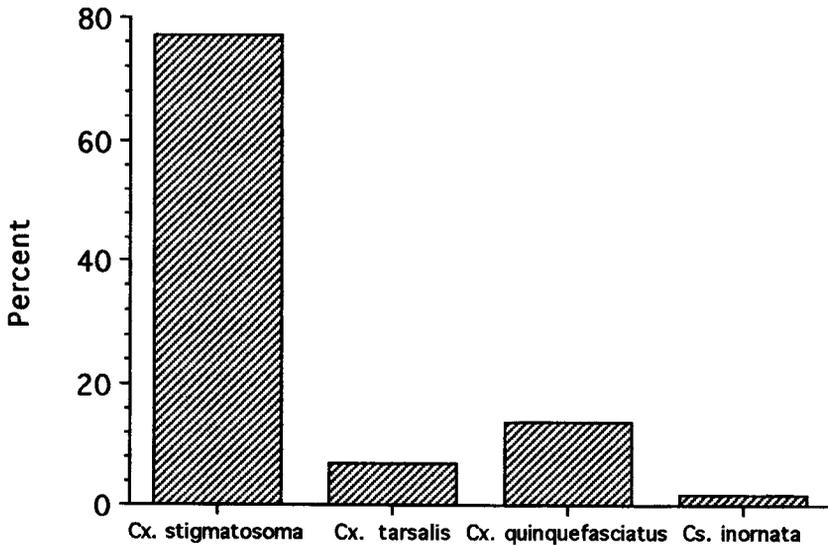
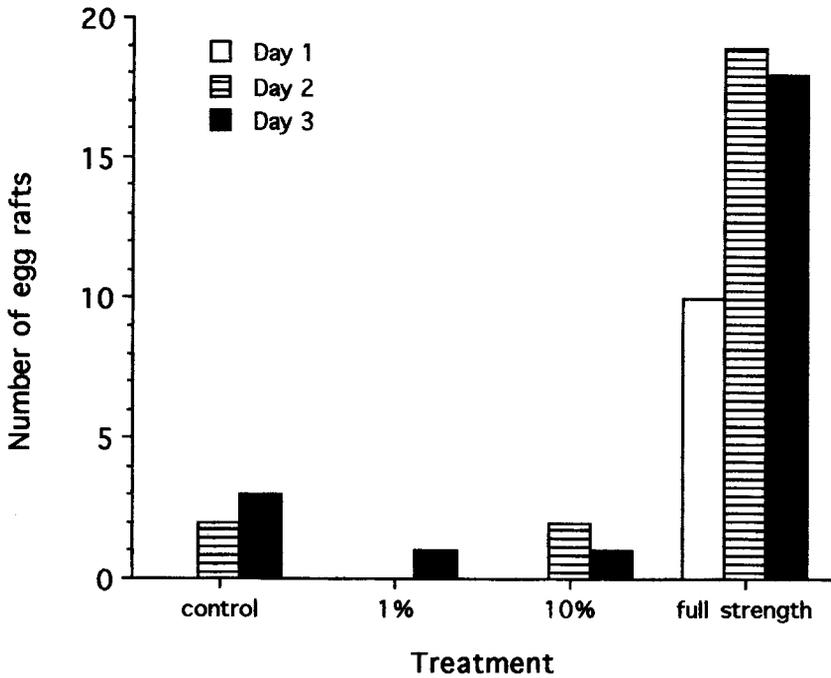


Fig 8. Oviposition by mosquitoes onto control (distilled water), and diluted and undiluted *Schoenoplectus californicus* infusion during the period November 6–10, 1995 (upper panel). The percent of egg rafts laid by species is shown in the lower panel.

*stigmatosoma*, 75%; *Cx. tarsalis*, 12%; *Cx. quinquefasciatus*, 6%; and *Cs. inornata*, 1% (Fig. 9).

**DISCUSSION**

Constructed wetland design significantly affected the size and age structure of larval mosquito populations. Larval population size of *Culex* spp. in one-phase marshes was larger than in 3-phase

marshes. Most of the differences in immature mosquito populations between the marsh types resulted from differences in the abundance of early larval instars (stages I and II) of *Culex* spp. In addition to the abundance of young larval instars in one-phase marshes being greater than in 3-phase marshes, larval populations in one-phase marshes contained proportionately more older instars than did populations in 3-phase marshes. The greater proportion

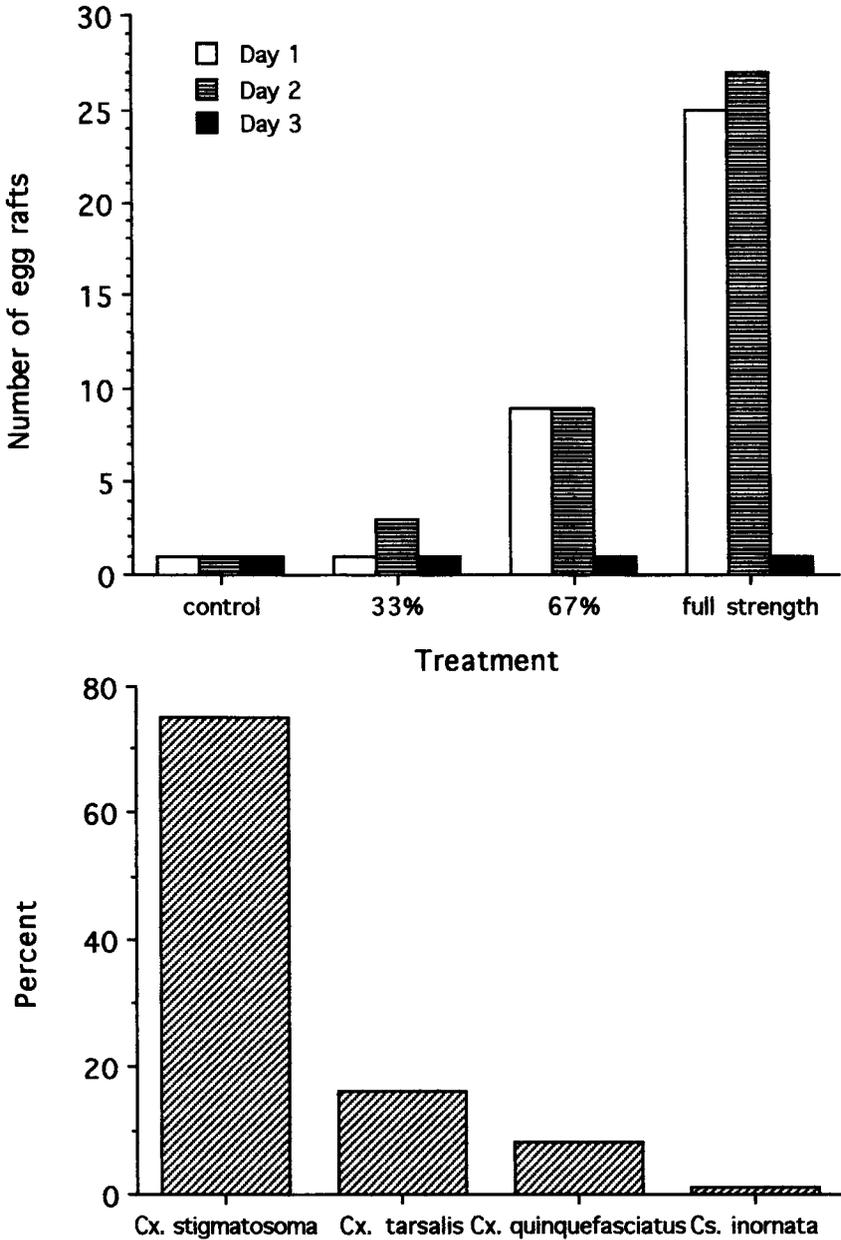


Fig 9. Oviposition by mosquitoes onto control (distilled water), and diluted and undiluted *Schoenoplectus californicus* infusion during the period November 14–17, 1995 (upper panel). The percent of egg rafts laid by species is shown in the lower panel.

of older larval instars in the populations in one-phase marshes throughout this study and the rarity of older larval instars in the populations in 3-phase marshes during August and September suggest that survivorship of larvae in one-phase marshes was better than in the 3-phase marshes.

Vertical estimates of larval mortality in one-phase vs. 3-phase marshes were generally in agreement with the differences in predator abundance in the 2 marsh types during June and July. Macroin-

vertebrate predators have been suggested to significantly affect larval mosquito populations, particularly several weeks after habitats are inundated (Mulla 1986, Walton et al. 1990). However, their effectiveness as mosquito control agents has been questioned because invertebrate predators are typically generalists (Zalom et al. 1978, Collins and Resh 1985). During late June and throughout July, notonectid populations (*Notonecta unifasciata* Guerin, *Notonecta irrorata* Say, and *Buenoa* sp.) in 3-

phase marshes were larger than in one-phase marshes. This difference might be the result of habitat preferences (for open water) by colonizing individuals of these planktonic predators and, consequently, by differences in population growth following the initiation of reproduction during the late spring. The experimental marshes were reflooded on June 1. Previous studies in 36-m<sup>2</sup> ponds found that notonectid abundance increased markedly at 3–4 wk after inundation (Walton et al. 1990). Notonectid populations in 3-phase marshes could have also been larger than in the one-phase marshes because the central ponds of the 3-phase marshes contained water prior to reflooding of the vegetation and were colonized earlier than the one-phase marshes.

The relationship between mortality rates of mosquito larvae and predatory macroinvertebrates was less evident in August and September, indicating that other factors presumably contributed to the low abundance of mosquito larvae during the 2nd half of the study. Predatory insect populations either declined or exhibited small fluctuations in population size. In contrast to the trends for predatory insects, mortality rates for larvae in one-phase marshes increased throughout the study. Mortality rates for larvae in 3-phase marshes increased from June through August. After early August, older larval instars were absent from samples taken in 75% of the 3-phase marshes. The disappearance of older larvae from samples taken in 3-phase marshes is indicative that larval survival was comparatively lower than in one-phase marshes. Although the decline in the abundance of *Culex* larvae was associated with increased predator abundance in both marsh types during June and July, either predators maintained the comparatively smaller larval populations at low levels during the late summer and early autumn or other, unmeasured factors contributed to the increase in larval mortality.

Declines in larval populations were not attributable to vertebrate predators. Mosquitofish (*Gambusia affinis* [Baird and Girard]) were unexpectedly found in the outflow of one of the 3-phase marshes (research cell 1). Mosquitofish had been stocked into the vegetation nursery cell adjacent to research cell 1. The conditions in the research cells were stressful for the mosquitofish, as sampling indicated that the fish were restricted to the outflow and the population remained small (<1 fish/trap) throughout the summer and autumn. Mosquito larval populations in research cell 1 were of intermediate size as compared to the other 3-phase marshes. The stressful conditions of both marsh types for fish is further substantiated by the failure of introductions of the 3-spined stickleback (*Gasterosteus aculeatus* L.) into both one-phase and 3-phase marshes (Walton et al. 1996).

We view the mortality estimates (Fig. 3B) as indicative of the general trends in mortality rather than precise estimates of larval mortality. For a por-

tion of this study when larval mosquito populations were abundant, mortality rates for larvae in 3-phase marshes were 2 or more times those estimated for larvae in one-phase marshes. The accuracy of the mortality estimates is dependent on sample size (Aksnes and Ohman 1996). Based on simulation studies carried out by Aksnes and Ohman (1996), mortality estimates are reasonably accurate during the first several weeks of our study when 300–15,000 larvae/research cell were counted. The accuracy of mortality estimates declined appreciably after week 5 when <30 larvae/research cell were collected and older larval instars (stages III and IV) were not present in most samples from 3-phase marshes.

In addition to factors causing larval mortality (Walton et al. 1990), differential oviposition based on differences in organic enrichment has been shown to influence the distribution of *Culex* mosquitoes (Beehler and Mulla 1993). The importance of differences in the attractiveness of the 2 marsh types to gravid female mosquitoes in causing the differences observed in larval abundance between one-phase and 3-phase marshes is, however, uncertain. Although the oviposition study using filtered water from the 2 marsh types (experiment 1) demonstrated that individual cells differed significantly in their attractiveness to gravid females, this experiment did not conclusively demonstrate a significant effect of marsh type. Oviposition was greatest onto water for research cell 6. Larval numbers in cell 6 were consistently greatest throughout the study. Tubs containing filtered marsh water were placed adjacent to research cells in early October. Oviposition tubs might have been competing for gravid females with the research cells when the differences in attractancy between the marsh types were comparatively small.

Oviposition experiments using bulrush infusions clearly showed that gravid females could distinguish among treatments in logarithmic and linear dilution series and that the full-strength bulrush infusion was most attractive to *Culex* females. Prior work has shown that gravid female *Cx. quinquefasciatus* and *Cx. tarsalis* can distinguish among treatments in a logarithmic dilution series of organic infusions (Millar et al. 1992, Isoe et al. 1995). The fact that gravid mosquitoes distinguished between the full-strength infusion and the full-strength infusion diluted in volume by one-third (67% treatment) is surprising. If differences in the attractancy of marsh types were related to differences either in the surface area covered by decaying *S. californicus* or in the volume of the 2 marsh types, then the difference in the intensity of oviposition in these experiments suggests that gravid females might be able to distinguish among marsh types. The 3-fold difference in mosquito oviposition between the full-strength and 67% full-strength treatments in experiment 3 is approximately the same difference observed in average larval densi-

ties between one-phase and 3-phase marshes during the middle and late summer. Even though the decaying plant biomass and incubation time were chosen to approximate plant density and residence time of water in the research cells, it is probably too simplistic to attribute the differences of population size of young larvae in the 2 marsh types to differences in oviposition attractancy. Other biotic and abiotic factors in addition to differential oviposition caused by differences in the amount of decaying vegetation between marsh types might have enhanced populations of young larvae in one-phase marshes as compared to 3-phase marshes.

Bulrush infusions show promise for arbovirus surveillance of *Cx. stigmatosoma* and *Cx. tarsalis*. In California, *Cx. tarsalis* and *Cx. stigmatosoma* are naturally infected with St. Louis encephalitis (SLE) virus (Reeves 1990). *Culex tarsalis* is thought to be the primary vector of SLE in California (Reeves and Milby 1990, Reisen et al. 1992) and is readily sampled by standard methods. *Culex stigmatosoma*, based on laboratory studies, may be important in the horizontal maintenance of SLE in southern California, but is often difficult to trap by standard methods (Reisen et al. 1992). Collection of parous (gravid) mosquitoes by oviposition traps (Reiter 1983, 1986) is one of the most effective arbovirus surveillance tools. Infusions of fermented organic matter have been used extensively as mosquito oviposition attractants and stimulants in population monitoring and arbovirus surveillance programs (see review in Isoe and Millar 1995). Even though few *Cx. stigmatosoma* adults were collected by CO<sub>2</sub>-baited trapping, larvae were abundant in the research cells and the majority of egg rafts collected in the oviposition experiments were of *Cx. stigmatosoma*. Bulrush infusions clearly stimulate oviposition by *Cx. stigmatosoma* and are also attractive or stimulatory to *Cx. quinquefasciatus* and *Cx. tarsalis*.

Interestingly, *Cx. erythrothorax* was not attracted to bulrush infusions in open tubs. This result is surprising because of this mosquito's documented developmental site preference for bulrush (tule) marshes (Bohart and Washino 1978). The large host-seeking population around the research cells throughout autumn and the persistence of the population through December suggest that gravid females were present during the oviposition studies. Gravid female mosquitoes use a combination of physical and chemical cues to choose oviposition sites (Benzon and Apperson 1988, Bentley and Day 1989). Physical factors such as height of vegetation and habitat size may play a particularly important role in the oviposition behavior of *Cx. erythrothorax*.

The potential of these marshes for mosquito production is likely much greater than is indicated by the dip samples. One might not necessarily expect a strong association between the species composition of the larval and host-seeking populations of

mosquitoes because of species-specific differences in dispersal tendencies, female longevity, attractancy to CO<sub>2</sub>, and so on. However, the size of the host-seeking population and the known developmental site preference for tule marshes of *Cx. erythrothorax* (Bohart and Washino 1978) suggest that our sampling underestimated the larval population of this species. Preliminary studies using emergence traps (Workman and Walton, unpublished data) indicate that the immature populations of *Cx. erythrothorax* in the marshes are larger than those of *Cx. tarsalis* and that mosquito production is concentrated in the vegetated regions of the cells. The difference in mosquito abundance between the 2 marsh types is even greater than is indicated by Fig. 2 because, on a per marsh basis, one-phase marshes contain approximately one-third more vegetated surface area than do 3-phase marshes.

Although the interrelationship among factors influencing mosquito populations in wetlands is complex, the interaction between naturally occurring predators and oviposition stimulation are probably important factors influencing larval populations of *Culex* mosquitoes. Three-phase marshes are comparatively more efficient at removing nutrients from secondary treated wastewater (USBR, NBS, and EMWD 1994<sup>1</sup>) and, following a die-off of *S. californicus*, support smaller mosquito populations than do one-phase marshes. Additional mosquito abatement may be required because naturally occurring mosquito predators do not limit mosquito production from hypereutrophic, constructed wetlands. An understanding of how vegetation type and natural cycles affect these systems, and how these factors impact our ability to use biological control agents for mosquitoes, is clearly needed. Our studies in the small marshes suggest that the physical and chemical conditions of the experimental constructed wetlands are stressful to larvivorous fishes (Walton et al. 1996). In addition to the high oxygen demands by bacterial populations utilizing decaying plant matter and the organic matter in secondary treated effluent, the lodging of dead, decaying *S. californicus* will restrict water flow to particular regions in the marsh and thereby limit the effectiveness of water-borne larvicides such as insect growth regulators and mosquito-specific bacteria. Thick accumulations of dead bulrush will also impede the penetration of downed vegetation by pelletized larvicide formulations. To the extent that the small experimental marshes accurately indicate how the larger ecosystems will function, constructed wetlands have an enormous potential for mosquito production.

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