

## EFFECTS OF ORGANIC ENRICHMENT ON TEMPORAL DISTRIBUTION AND ABUNDANCE OF CULICINE EGG RAFTS

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**ABSTRACT.** The effect of organic enrichment of the larval habitat on mosquito oviposition was determined in 2 replicated field experiments. In the first study, *Culex stigmatosoma* and *Culex quinquefasciatus* oviposition (as measured by the number of early instar larvae) reached a maximum peak soon after flooding and then declined to a substantially lower equilibrium level. A group of ponds that later received supplemental organic matter had a significant increase in oviposition soon after the additional organic matter was added. In the second experiment, a similar ovipositional pattern was noted by the sampling of egg rafts along transects and determining larval abundance by the dipping technique. In the second experiment a mixture of *Culex stigmatosoma* and *Culex tarsalis* larvae was collected.

### INTRODUCTION

A wide range of mosquito larval habitats have been described and classified into several broad categories (e.g., containers, impoundments, floodwater). However, the ovipositional cues for the selection of these specific sites by gravid mosquitoes are not well understood. A number of laboratory studies have identified various chemical substances that influence oviposition site selection, but to date little field data are available (Bentley and Day 1989).

Organically enriched temporary water sources in southern California produce large numbers of *Culex* mosquitoes (*Culex quinquefasciatus* Say, *Culex tarsalis* Coq., *Culex stigmatosoma* Dyar), and in many areas, these mosquitoes have adapted to their larval habitats in the urban/suburban environment (i.e., water storage or reclamation ponds, duck club ponds, underground drainage systems, poorly maintained swimming pools, and animal watering vessels). Mulla (1990) noted that the numbers of larval *Culex* mosquitoes breeding in some of these temporary habitats reached a peak soon after flooding and declined precipitously to a lower level. One of the factors attributed to this decline was a decrease in attractiveness of the habitat to ovipositing mosquitoes.

Beehler and Mulla (1993) demonstrated a temporal decline in the number of *Culex* larvae in a temporary habitat. They showed this decline to be due primarily to the quality and quantity of oviposition attractants, which peaked 2 to 3 wk after flooding and then declined markedly. In addition to the changes in oviposition attractants in the temporary habitat, Walton and Mulla (1991) showed that macroinvertebrate populations peaked after this 2- to 3-wk period of maximum attractancy. Many of these invertebrates are predaceous on mosquito larvae and undoubtedly play a major role in the regulation of larval numbers.

In the present study, we wanted to determine

the effect of oviposition success on larval distribution patterns under conditions where predators have had sufficient time to increase. We also wanted to determine the effect of additional organic enrichment of ponds (to increase ovipositional attractancy) that had undergone an increase and subsequent decline in mosquito larvae.

### MATERIALS AND METHODS

Two experiments to determine the effect of organic enrichment on the oviposition success of *Culex* mosquitoes were conducted at the Aquatic and Vector Control Research Facility in Riverside, CA, in replicated wood-sided ponds (27 m<sup>2</sup>) that were free of vegetation.

*Experiment 1:* Ten ponds were flooded with canal water to a mean depth of 30 cm on June 30, 1991. Water level was maintained with a float valve system. Four kilograms of rabbit pellets (Hare Raiser Pellet 16, Poultryman's Cooperative Assoc., Atascadero, CA) were added to each pond at the time of flooding. Three days post-flooding, all ponds were treated with bifenthrin (0.5 g AI/ha) to control a predaceous tadpole shrimp (*Triops longicaudatus* [Notostraca: Triopsidae]). Bifenthrin, at the low rate used, has no effect on mosquito larvae when used before significant oviposition by mosquitoes occurred (Mulla et al. 1992).

To assess the effect of organic enrichment on oviposition, mosquito larval densities were monitored twice weekly with a 350-ml dipper. Five dips were taken per pond with one dip in each of the corners and one dip taken along the north side. Water temperature was recorded with a maximum-minimum thermometer. Subsamples of larvae were returned to the laboratory, reared to the 4th instar and identified to species.

After 3 wk of flooding when mosquito larval abundance had reached a peak, 5 ponds were

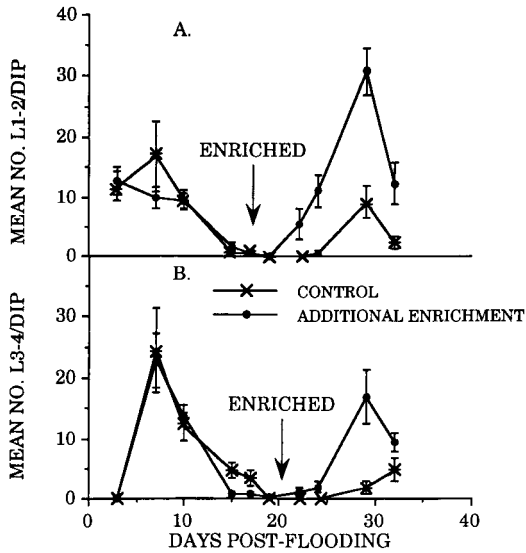


Fig. 1. Abundance of early (A) and late (B) instar *Culex quinquefasciatus* and *Culex stigmatosoma* larvae in ponds receiving one or 2 organic enrichments.

selected using a randomized block design and additional rabbit pellets (4 kg) were added. The other ponds were left without additional enrichment as controls. Ponds were sampled as above for the next 2 wk.

Larval samples were divided into 2 groups, early instars (L1–2) and late instars (L3–4), and counted. Data from ponds that received the additional organic enrichment and the control ponds were transformed to square roots ( $\sqrt{x}$ ), an appropriate transformation where the variance increases with an increase in the mean (Box et al. 1978). The numbers of larvae sampled in the ponds that received the additional enrichment were compared to the numbers of larvae in the control ponds using multiple regression with dummy variables to allow for comparison before and after the addition of additional organic enrichment (Chatterjee and Price 1977). In 4 of the ponds, the water flow from the float valve system appeared greater than in the other 6 ponds. This increase in water flow was attributed to higher percolation rates. Any block effect attributed to these 4 high flow ponds was also considered using dummy variables in the regression analysis.

**Experiment 2:** Nine experimental ponds were organically enriched and flooded in Riverside as in the first experiment on June 26, 1993. The ponds were treated 3 days postflooding with bifenthrin (0.5 g AI/ha) to control tadpole shrimp and additional organic enrichment was added to 4 of the ponds 14 days postflooding using a randomized block design. Larval dip samples were

Table 1. Multiple regression analysis ( $\sqrt{x}$ -transformed data) of the first field experiment.

Source	Coefficient	P value
Intercept		
Early instars (L1–2)	3.19	
Late instars (L3–4)	2.08	
Density before additional enrichment		
Early instars	–0.12	0.77
Late instars	–0.22	0.62
Density after additional enrichment		
Early instars	1.93	0.004
Late instars	1.29	0.05
Day effect (change in density over time)		
Early instars	–0.13	0.001
Late instars	–0.03	0.37
High water flow effect		
Early instars	0.53	0.099
Late instars	0.34	0.32

Regression summary: Early instars;  $df = 5, 104$ ;  $F = 7.41$ ;  $P = 0.0001$ ;  $R^2 = 0.28$ . Late instars;  $df = 5, 104$ ;  $F = 1.38$ ;  $P = 0.24$ ;  $R^2 = 0.06$ .

taken 6, 12, 14, and 16 days postflooding using the protocol in the first experiment. In contrast to the first experiment, oviposition was also measured directly by counting egg rafts along a 11-m  $\times$  4-cm transect located along the north and west sides of each pond using an aquarium net (Beehler and Mulla 1993). Subsamples of egg rafts were returned to the laboratory and reared to 4th-instar larvae for identification. Data analysis procedure was similar to the previous study using blocking variables.

## RESULTS AND DISCUSSION

**Experiment 1:** The temporal larval distribution pattern followed a predictable course after flooding. Within 1 wk after flooding the number of early instars (L1–2) reached a maximum peak (Fig. 1A). The number of late instars (L3–4) also increased rapidly, lagging only slightly behind the early instars (Fig. 1B). This smaller than expected lag period is probably related to the high summer temperatures during the study period, with water temperatures ranging from 23.3 to 33.3°C. After this peak, the larval populations declined markedly as expected. During this initial period (before adding the additional organic enrichment), there were no significant differences in both early and late-instar larval numbers sampled from both groups of ponds (Table 1).

After adding the additional organic enrichment, the number of early instar larvae increased

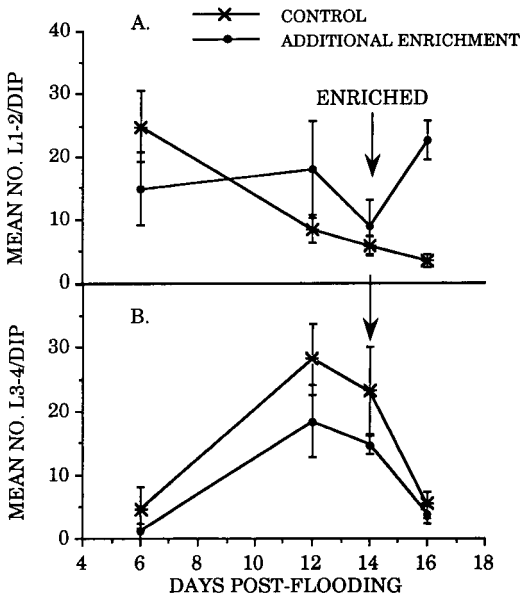


Fig. 2. Abundance of early (A) and late (B) instar *Culex stigmatosoma* and *Culex tarsalis* larvae in ponds receiving one or 2 organic enrichments.

in the ponds receiving the additional enrichment, indicating a corresponding increase in *Culex* oviposition (Fig. 1A). There was also a slight increase in the number of early instar larvae in the control ponds, although the ponds receiving additional organic enrichment had significantly higher ( $P = 0.004$ ) larval numbers (Table 1). The experimental ponds at Riverside are separated by 3 m from each other, and therefore ponds that received additional enrichment were very close to those that did not receive additional organic matter. Any volatile attractants produced by microbial degradation in the ponds that received additional organic enrichment might have an effect on nearby ponds. However, close-range attractants and oviposition stimulants resulted in significant differences between the 2 groups of ponds. A similar pattern was noted with the late-instar larvae. Again, significantly more late-instar larvae ( $P = 0.05$ ) were collected in the ponds that received additional organic enrichment (Table 1). Ponds that had higher water inflow did not have significantly different numbers of early ( $P = 0.09$ ) or late- ( $P = 0.32$ ) instar larvae than did ponds with the lower flow.

Larval samples taken throughout this study showed about a 50:50 mixture of *Cx. quinquefasciatus* and *Cx. stigmatosoma*. The species composition did not vary between ponds receiving additional organic enrichment and ponds not receiving the additional enrichment.

*Experiment 2:* Larval abundance patterns were

Table 2. Multiple regression analysis ( $\sqrt{x}$ -transformed data) of the second field experiment.

Source	Coefficient	P value
<b>Intercept</b>		
Egg rafts	3.11	
Early instars (L1-2)	3.50	
Late instars (L3-4)	-9.27	
<b>Density before additional enrichment</b>		
Egg rafts	0.02	0.91
Early instars	0.03	0.95
Late instars	-0.90	0.06
<b>Density after additional enrichment</b>		
Egg rafts	1.84	0.0001
Early instars	2.99	0.005
Late instars	0.69	0.43
<b>Day effect (change in density over time)</b>		
Egg rafts	-0.14	0.001
Early instars	0.33	0.71
Late instars	2.41	0.004
<b>High water flow effect</b>		
Egg rafts	-0.30	0.10
Early instars	-1.18	0.29
Late instars	0.15	0.70

Regression summary: Egg rafts;  $df = 5,57$ ;  $F = 15.67$ ;  $P = 0.00001$ ;  $R^2 = 0.58$ . Early instars;  $df = 6,29$ ;  $F = 4.053$ ;  $P = 0.005$ ;  $R^2 = 0.46$ . Late instars;  $df = 6,29$ ;  $F = 11.33$ ;  $P = 0.0001$ ;  $R^2 = 0.71$ .

similar to those in experiment 1, but in this experiment oviposition was also measured directly. Six days postflooding, early instar larvae reached a maximum mean density and then the numbers began to decline until day 14 when 4 of the ponds received the additional organic enrichment (Fig. 2A). Before this enrichment was added there was no significant difference ( $P = 0.9$ ) in numbers of early instars sampled between ponds receiving the additional enrichment and the control ponds (Table 2). However, 2 days after additional enrichment the mean number of early instar larvae increased significantly ( $P = 0.005$ ), whereas the number of early instar larvae in the control ponds continued to decline (Table 2). Water temperatures during this experiment were also high (16.6–33.3°C), allowing for rapid larval development.

The populations of late-instar larvae reached a maximum peak on day 12 (before the additional enrichment was added) and declined for the rest of the study period (Fig. 2B). On each sampling date there was no significant difference, either before or after additional organic enrichment was added, in the mean number of late-instar larvae sampled in the 2 groups of ponds

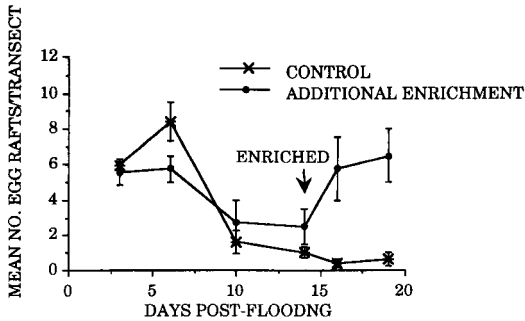


Fig. 3. Abundance of *Culex stigmatosoma* and *Culex tarsalis* egg rafts collected along a transect in ponds receiving one or 2 organic enrichments.

(Table 2). We cannot say if a second peak would have occurred in the late-instar larvae, because the experiment was terminated before the recently hatched early instar larvae could mature. If the study had continued longer, late-instar larvae may have shown an increase in density corresponding to the increasing number of early instar larvae. In contrast to the first experiment, ponds adjacent to those that received the additional organic enrichment did not have a noticeable increase in either early or late-instar larval density. On the other hand, increasing predator pressure may have reduced larval numbers, causing mortality in the larvae before they reached the later developmental stages.

The most accurate assessment of the effect of additional nutrient enrichment on the dynamic pattern of oviposition in experimental ponds comes from the direct measurement of oviposition intensity along a transect. About 1 wk after flooding, *Culex* oviposition in all ponds peaked (Fig. 3). During this period there was no difference in the number of egg rafts collected in any of the ponds (Table 2). Egg raft numbers declined and a group of the ponds were provided with additional organic enrichment on day 12. After this addition, there were significantly more ( $P = 0.0001$ ) egg rafts counted per transect in the ponds receiving the additional enrichment compared to the control ponds (Table 2). The number of rafts in the control ponds continued to decline until the end of the experiment, whereas the number of egg rafts in the ponds receiving additional enrichment continued to increase. As in the first experiment, there were no significant differences in egg raft or larval numbers between ponds with higher water flows and those with lower flows.

Subsamples of egg rafts sampled in this experiment and reared to the 4th instar were identified to be mostly *Cx. stigmatosoma* with a few *Cx. tarsalis* (<5%). The species composition did

not change in the ponds receiving additional enrichment or in the controls.

These experiments support the earlier results of Beehler and Mulla (1993) showing that the oviposition attractancy of temporary developmental habitats for *Culex* is dynamic, changing from highly attractive soon after flooding and then declining to a lower level of attractancy within a few weeks. The data presented here clearly show that this attractancy is the result of the availability of organic matter, which on microbial degradation produces oviposition attractants. The additional organic enrichment in some of the ponds made organic matter available again as a source of energy for microorganisms, producing oviposition stimuli leading to increased oviposition.

These results have important predictive implications for mosquito control as ponds such as dairy waste-water lagoons have a constant influx of organic material and therefore will produce high levels of oviposition attractants continuously (as long as organic matter is added). Mosquito breeding has been shown to decline when the influx of organic matter stops in these lagoons (Mulla, unpublished data). In other settings, such as the flooding of duck club ponds, mosquito populations will increase rapidly with the initial production of oviposition attractants. The number of mosquito larvae will then decline to lower levels as the limited amount of organic matter involved in the production of attractants is decomposed. In these situations, early treatment with larvicides will control the initial larval population peak that will produce adults before sufficient numbers of predator species exert regulatory pressure. Later, the relatively few mosquitoes that oviposit in the less attractive habitats may be adequately controlled by the increasing predator population.

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#### REFERENCES CITED

- Beehler, J. W. and M. S. Mulla. 1993. The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes. Proc. Calif. Mosq. Vector Control Assoc. 61:121–124.  
 Bentley, M. D. and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Annu. Rev. Entomol. 34:401–421.

- Box, G. E. P., W. G. Hunter and J. S. Hunter. 1978. Statistics for experimenters. An introduction to design, data analysis and model building. John Wiley and Sons, New York.
- Chatterjee, S. and B. Price. 1977. Regression analysis by example. John Wiley and Sons, New York.
- Mulla, M. S. 1990. Activity, field efficacy, and use of *Bacillus thuringiensis israelensis* against mosquitoes, pp. 134–160. In: H. de Barjac and D. J. Sutherland (eds.). Bacterial control of mosquitoes and blackflies. Biochemistry, genetics and applications of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus*. Rutgers Univ. Press, New Brunswick, NJ.
- Mulla, M. S., M. Zgomba, H. A. Darwazeh and J. D. Chaney. 1992. Efficacy and selectivity of two pyrethroid insecticides against the predator *Triops longicaudatus* (Notostraca: Triopsidae) and *Culex tarsalis* larvae. Bull. Soc. Vector Ecol. 17:51–56.
- Walton, W. E. and M. S. Mulla. 1991. Integrated control of *Culex tarsalis* larvae using *Bacillus sphaericus* and *Gambusia affinis*: effects on mosquitoes and nontarget organisms in field mesocosms. Bull. Soc. Vector Ecol. 16:203–221.