Larval behavior of four *Culex* (Diptera: Culicidae) associated with treatment wetlands in the southwestern United States

Parker D. Workman[1] and William E. Walton[*]

Department of Entomology, University of California, Riverside, CA 92521-0314, U.S.A.
[*]Corresponding author

Received 4 December 2002; Accepted 12 May 2003

ABSTRACT: The diving behavior, aggregation, horizontal distribution under two food regimes, and response to change in ambient light level were studied for fourth instars of four *Culex* species in the laboratory. Under low food conditions, *Cx. erythrothorax* larvae dived less frequently, exhibited greater aggregation, and had stronger preferences for the corners of the observation chamber than did larvae of three congeners (*Cx. tarsalis*, *Cx. quinquefasciatus*, and *Cx. stigmatosoma*). Under high food conditions, larvae of all species were more sedentary than under low food conditions; however, the behavior of *Cx. erythrothorax* larvae changed comparatively little with changes in food abundance. *Culex erythrothorax* was the most responsive to disturbance (an 11% change in ambient light level), whereas *Cx. tarsalis* larvae did not respond to small changes in ambient light level. The behavior of *Cx. erythrothorax* larvae differed significantly from the three congeners studied. Species-specific behavioral differences of larvae may have contributed to the discrepancies of species relative abundance in larval surveys versus emerging adult mosquito collections at constructed wetlands noted in previous studies. ***Journal of Vector Ecology* 28 (2): 213-228. 2003.**

Keyword Index: Culex, larval behavior, mosquitoes, constructed treatment wetlands.

INTRODUCTION

Surveillance of vector abundance and vector-borne pathogen activity provides information critical to decision-making for the management and prevention of vector-borne diseases (Rose 2001). The abundance of mosquito larvae in dipper samples is used commonly to determine whether abatement procedures need to be carried out. While the threshold larval mosquito abundance at which abatement is carried out differs among agencies and circumstances (Tennessen 1993, Collins and Resh 1989, Knight et al. 2000), an accurate assessment of mosquito populations is particularly important when mosquito developmental sites are located near areas of human habitation and mosquito-borne pathogen activity has been detected. The proliferation of man-made wetlands in urban and suburban settings and the potentially high level of mosquito production from some wetlands used to reduce the concentration of nutrients in municipal wastewater (Walton et al. 1998) indicate that mosquito populations need to be monitored closely.

Four *Culex* species are commonly found at constructed treatment wetlands receiving municipal wastewater in the southwestern United States: *Culex tarsalis* Coq., *Cx. erythrothorax* Dyar, *Cx. quinquefasciatus* Say, and *Cx. stigmatosoma* Dyar. *Culex tarsalis* is a competent vector of arboviruses such as St. Louis encephalitis (SLE) virus and western equine encephalomyelitis (WEE) virus (Reeves and Hammon 1962) and is the greatest public health concern among the four species. Although *Cx. erythrothorax* is a comparatively refractory vector of SLE and WEE viruses (Meyer et al. 1988, Reisen et al. 1992), recent laboratory studies indicate *Cx. erythrothorax* is a competent vector of West Nile virus (Goddard et al. 2002). *Culex quinquefasciatus* is thought to be an important vector of encephalitides in urban situations, particularly in the eastern and central U.S. (Reisen and Reeves 1990). Even though *Cx. stigmatosoma* is a very efficient vector of encephalitis viruses, its narrow host range (Reisen and Reeves 1990) suggests that this species is an important

[1]Present address: Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, MA 02115, U.S.A.

vector that maintains infections in avian populations but is probably not important for transferring viruses to humans.

Culex erythrothorax and *Cx. tarsalis* predominate in the adult mosquito populations emerging from constructed treatment wetlands and also predominate in carbon dioxide-baited suction trap collections from these wetlands in the arid southwestern U.S. (Walton and Workman 1998, Walton et al. 1998, Workman and Walton 2000). Both species increase in abundance as emergent vegetation colonizes and fills the shallow water (<1 m depth) zones of new or renovated treatment wetlands (Walton 2002, Keiper et al. 2003).

The spatial distribution of *Cx. erythrothorax* emergence was related to a resource gradient along the path of water flow and was associated strongly with emergent vegetation, but adult mosquito emergence was not restricted to the comparatively deep, central zone of a constructed treatment wetland (Workman and Walton 2000). Even though *Cx. erythrothorax* adult production occurred throughout the vegetation of a treatment wetland and exceeded that of congeners by more than an order of magnitude on most dates, late instar *Cx. erythrothorax* were comparatively rare (< 5% of the total larvae collected) in dipper samples (Walton and Workman 1998).

To assess whether species-specific behavioral differences contributed to an under-representation of late instars of *Cx. erythrothorax* in larval surveys and how larval behavior may change along gradients of potential larval resources found in constructed treatment wetlands, the larval behavior of four *Culex* species (*Cx. erythrothorax*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis*) that inhabit constructed treatment wetlands in southern California was compared. The behavior (diving behavior, aggregation, and horizontal distribution) of fourth instars was examined in the laboratory under two food regimes and in response to a disturbance.

MATERIALS AND METHODS

Larval collection and rearing

All larvae used in behavioral observations were the first generation progeny of wild adults inhabiting a constructed wetlands research complex located in San Jacinto, California. Host-seeking adults were collected using carbon dioxide-baited suction traps. *Culex erythrothorax* and *Cx. tarsalis* larvae were obtained from egg rafts laid by field-caught females that were offered blood meals from mice or 1 day-old chicks, respectively, in the laboratory. *Culex quinquefasciatus* and *Cx. stigmatosoma* were collected as egg rafts from black plastic tubs (length × width × depth: 47 cm × 36 cm × 18 cm) baited with bulrush (*Schoenoplectus californicus* (Meyer) Soják or *S. acutus* (Muhl. ex. Bigel.) Löve and Löve) infusions (Walton and Workman 1998). Egg rafts obtained through both methods were separated into 200 ml (6 oz) Sweetheart waxed ice cream cups for rearing. Larvae were reared at 31± 2 °C, on a light:dark cycle of 14:10 h, and fed a mixture of finely ground mouse chow and brewer's yeast (3:1). Upon reaching the fourth instar, larvae were identified using Bohart and Washino (1978).

Larval videotaping

Five larvae from a single egg raft of each species in the first or second day of the fourth instar were chosen at random for each videotaping session. Eight 45-min videotape sessions were replicated for each of the four *Culex* species studied. Each taping session was comprised of three 15-min periods: diving behavior under low food conditions, diving behavior associated with a light/shadow stimulus, and diving behavior under high food conditions. One group of *Cx. erythrothorax* larvae pupated on the day after taping and was removed from subsequent analyses.

Larval behavior was videotaped using a Sharp Hi8 Viewcam (Model VL-H410V) recording at a rate of 30 frames/s. The camera was operated by remote control and was positioned 2.8 m from the aquarium in a room isolated from the main laboratory. The side of the aquarium filled the camera's field of view and all larvae were visible.

During the course of the study, one of two acrylic aquaria were used: an aquarium (14.0 cm × 0.9 cm × 11 cm) with a clear front and back and blackened sides and bottom that was placed against a white backdrop, or an aquarium (14.0 cm × 2.6 cm × 12.2 cm) with a clear front, white sides and back, and a black bottom. The latter aquarium allowed better resolution of the larvae during tape analysis and was used in approximately half of the taping sessions for each species. The aquaria were filled to a depth of 10 cm with aged (aerated 2 wk), filtered (Supor® 200 membrane filter; pore size = 0.22 mm) tap water that was changed between videotaping sessions. The aquarium was illuminated using a combination of a 20 W full spectrum fluorescent light bulb (Fluker Laboratories #RS1-24-20W) suspended 20 cm above the aquarium, a 75 W incandescent light bulb (Phillips) suspended 30 cm above the top of the aquarium, as well as six overhead fluorescent lights (40 W Sylvania Super Saver Cool White). Ambient light levels at the surface of the water during taping were 786 lm/m^2.

Larval behavior under low food conditions (<1 X 10^3 particles/ml) was recorded first in all sessions. Larvae

were placed into aged, filtered tap water and were allowed ten min to acclimate in the aquarium before their behavior was recorded.

Diving behavior associated with a light/shadow stimulus was elicited by decreasing light levels by 11% (~89 lm/m^2) from ambient levels in the room. This stimulus was used to approximate a change of light level experienced by larvae inhabiting vegetated zones near the vegetation-open water interface of wetlands during larval sampling using a dipper. It is equivalent to a proportionate change in light level when a white plastic, 350 ml dipper is held 15 cm directly above the water surface at a position in the shade at 7.5 cm from the sunlight-shade interface at 12:00 h on a summer day (measured using an upward-facing flat light sensor: Extech Instruments light meter (#6603-4097); Whatman International Ltd., Maidstone, UK). Light levels in the laboratory were altered by quickly flashing (off and on in <1 s) the overhead fluorescent lights. The light stimulus episodes were taped immediately following exposure of larvae to low food conditions and were comprised of five successive stimuli at three min intervals.

Larval behavior in high food conditions was recorded after the addition of 0.5 ml of an activated yeast solution (0.026 g (small aquarium) or 0.075 g (large aquarium) Fleishman's Yeast /ml of water: ~3.5 X 10^8 particles/ml). After addition of the yeast suspension, the media in the aquarium was thoroughly mixed by pumping of the pipette used to add the yeast approximately 15 times. Larvae were allowed 10 min to acclimate before taping commenced.

Videotape analysis

Larval mosquito behavior was analyzed using a Sony EVS-5000 Hi8 videocassette recorder and a personal computer equipped with Image Pro® image analysis software (Media Cybernetics, Silver Spring, MD). The position of each larva was tracked by following the larval head capsule. Any movement that caused the siphon to lose contact with the air/water interface was considered a dive.

For the analysis of diving behavior associated with low and high food conditions, episodes were analyzed for the total number of dives (dive frequency), dive type and dive length. Dives were assigned to one of two categories: transitional or deep dives. Transitional dives were defined as those dives that were shorter than two seconds, were no deeper than 1 cm, and were predominantly horizontal in nature. Starting and ending points, as well as total dive time, were measured for the transitional dives.

Deep dives had a maximum depth > 1 cm and were generally ≥ 2 s in duration. During these dives, the larval siphon broke contact with air/water interface, the larva dived to a maximum depth, and either immediately began ascending from that depth or maintained that depth with or without lateral movement before ascending. For deep dives, the starting and ending points, the point of maximum depth, and the length of time for each aspect of the dive were recorded.

To determine whether larvae aggregated and preferred particular regions of the aquarium (e.g., corners), position of larvae at the water surface was measured under low and high food conditions. Images were sampled every 30 s within each episode and the horizontal position of each larva at the water surface was measured relative to the left wall of the aquarium. The minimum distance between each larva and its nearest neighbor at the water surface was determined for each image analyzed.To assess whether larvae had a preference for the margins of the aquarium versus the more lighted areas nearer the middle of the aquarium, the aquarium was divided into 4 vertical sections: corners (10% of the water surface at the edges) and three equally-sized sections (the center 30% of the water surface; the near center, 15% of the water surface on each side of the center zone; and the far center, 15% of the water surface on each side of the combined center and near center zones). The number of larvae in each vertical section of the aquarium was scored and summed for each taping session.

Analysis of behavior associated with the light stimulus considered the number of larvae responding to the change in light level and the duration of each dive.

Statistical analyses

Differences of larval mosquito behaviors among species were tested using a multiple within-subject factor (food level, behavior) repeated-measures multivariate analysis of variance (MANOVA). A subset of the behaviors was analyzed because multicollinearity and missing values (e.g., dive times could not be calculated for all replicates under high food conditions because larvae did not dive) reduced the size of the full data matrix, and transformation did not normalize the distributions of some behaviors under high food conditions. Three behaviors (log-transformed number of deep dives, log-transformed aggregation, and arcsine-transformed square root proportion of larvae in the corners of the observation chamber) were analyzed concurrently by MANOVA and the canonical correlations among variables (Tabachnick and Fidell 1996, Scheiner 2001) were examined using SYSTAT (ver. 9.0). Because only two food levels were assessed within subjects, standard multivariate statistics were not

generated for some tests; *F* statistics are provided for those tests. Repeated-measures MANOVAs for each of the three behaviors (between-subject factor: species; within-subject factor: food level), as well as for the duration of deep dives, were also carried out. Differences among species were tested by profile analysis using contrasts (von Ende 2001).

Transitional diving activity frequently did not occur at the high food level and the distribution of transitional dives per session was not normal. Differences in the number of transitional dives carried out by the four *Culex* species were analyzed within each food level using a non-parametric one way ANOVA (Kruskal-Wallis test on ranked data; α=0.007) followed by Dunn's test for differences between species (Fox et al. 1995).

The horizontal distribution of larvae in the aquarium was analyzed using a Chi-square test. The null hypothesis assumed a random distribution of larvae across the sections of the aquarium (i.e., 10% occurrence near the corners and 30% occurrence in each of the three more central zones).

The median proportion of larvae responding to the light stimulus was compared across species using a non-parametric ANOVA. Attenuation of the response by larvae to the light stimulus was tested for each species using Cochran's Q-test (Sokal and Rohlf 1995). If the larvae were acclimating to the light stimulus, then the proportion of larvae responding would be expected to decrease across time.

To assess whether larvae responding to the light stimulus remained submerged longer than did larvae diving naturally, dive times of larvae responding to the stimulus were compared to dive times for larvae under low food conditions where light levels remained constant. A two-way repeated-measures ANOVA was carried out on log-transformed dive times. The main effects of species and light condition, and their interaction, were examined. Multiple pairwise comparisons between species dive times were made using Tukey's test.

The relationships among composite behavioral variables were compared among the four *Culex* species by ordination using SYSTAT. Eleven behavioral variables were included in the principal components analysis (PCA). For each food level, the mean for dive frequency (log-transformed), number of deep dives (log-transformed), and the proportion of larvae near the corners of the aquarium for each 15-min taping session were included in the PCA. Because larvae did not dive under high food conditions in many taping sessions, dive times at the high food level could not be calculated for all taping sessions and were excluded from the ordination. The mean log-transformed dive time of larvae under low food conditions, the median minimum distance between larvae at high and low food levels, the median number of transitional dives, and the proportion of larvae responding to a change in ambient light level for each taping session also were variables in the analyses. Data within each behavioral category were standardized to have a mean = 0 and unit variance. A maximum of three factors (principal component axes with eigenvalues > 1) were computed. Species centroids in three-dimensional space were calculated using the means of factor scores derived for each taping session. Calculation of 95% confidence ellipses was done by centering the ellipse in *x-y* space on the sample means, using the standard deviations on each axis to determine the major axes, and using the Pearson correlation coefficient to determine the orientation of the ellipse (SYSTAT, 1999). Ellipses for each pair of PCA axes were used to construct ellipsoids for species.

The principal component scores for the three axes were compared among species using MANOVA. Pairwise comparisons of least squares means among species were carried out using the Bonferroni adjustment following significant univariate ANOVAs (α = 0.017) for scores on each of the uncorrelated principal components.

RESULTS

Larval mosquito behavior differed significantly between food levels and among the three behavioral categories considered concurrently (Table 1). Although the between-subject main effect was not significant, all interactions were statistically significant indicating that food levels did not similarly affect the behaviors and that the behaviors of the four *Culex* species did not differ consistently between food levels.

The canonical loadings of the variables on the two significant canonical variates (Bartlett test of residual correlations, P <0.05) indicated that behaviors under low food conditions and a subset of behaviors under high food conditions were significantly related to the variates. The proportion of generalized variance (i.e., multivariate R^2) of the species correlation matrix explained by the correlation matrix of the behavioral variables was 0.93. Behaviors under low food conditions [canonical loadings: aggregation (i.e., minimum distance between larvae), -0.61; deep diving behavior, -0.89; propensity to reside in the corners of the observation chamber, 0.81] and propensity to reside in the corners of the observation chamber under high food conditions (0.76) were strongly associated with the first canonical covariate (canonical correlation: r_c = 0.89). This variate was positively associated (canonical loadings ≥ 0.45) with species except *Cx. erythrothorax* and was indicative of widely

Table 1. MANOVA of larval behaviors of four *Culex* species under two food regimes.

Source	MS	df	Statistic	Value	P
Between-subjects					
Species	0.102	3	F	1.388	0.268
Error	0.074	27			
Within-subject					
Food	12.637	1	F	145.991	<0.0005
Food × species	0.254	3	F	2.936	0.051
Error	0.087	27			
Behavior		2, 26*	Pillai's trace	0.958	<0.0005
Behavior × species		6, 54	Pillai's trace	0.692	0.001
Behavior × food		2, 26	Pillai's trace	0.760	<0.0005
Behavior × food × species		6, 54	Pillai's trace	0.651	0.001

*Numerator df, denominator df.

spaced larvae that dived frequently under low food conditions. The second canonical variate ($r_c = 0.78$) was associated predominantly with aggregation under low food conditions (-0.69) and was highly correlated with *Cx. erythrothorax* (0.97).

Number of dives

The mean number of dives (all dives: Figure 1) did not differ significantly among the four *Culex* species across food levels ($F_{3,27} = 1.05$, $P = 0.387$); however, the within-subject food level effect ($F_{1,27} = 102.00$, $P < 0.0005$) was significant. When the larvae were in high food conditions, the four *Culex* species had reduced dive frequencies (Figure 1). The dive frequency of *Cx. erythrothorax* larvae under high food conditions was 43% of that observed under low food conditions. Dive frequencies of the other *Culex* species under high food conditions were ≤11% of the dive frequencies observed under low food conditions.

The number of deep dives per 15-min interval (Figure 2A) differed significantly between food levels ($F_{1,27} = 102.18$, $P<0.0005$). The number of deep dives did not differ significantly among species across food levels ($F_{3,27} = 2.06$, $P = 0.129$), but the food level by species interaction was significant ($F_{3,27} = 2.98$, $P=0.05$). In high food conditions, deep dive frequency did not vary significantly among species (contrasts, $P>0.05$). Under low food conditions, *Cx. erythrothorax* dived less frequently than did the other species (Figure 2A; contrasts: *stigmatosoma* > *quinquefasciatus*, *tarsalis* > *erythrothorax*).

The number of transitional dives (Figure 2A) did not differ significantly among the four *Culex* species ($F_{3,27} = 1.52$, $P<0.233$). Under low food conditions, transitional dives were carried out more frequently than under high food conditions (food level: $F_{1,27} = 53.18$, $P<0.0005$). The number of transitional dives carried out by *Cx. stigmatosoma* and *Cx. tarsalis* in the high food environment was significantly lower than in the low food environment (species × food level: $F_{3,27} = 3.85$, $P<0.021$;

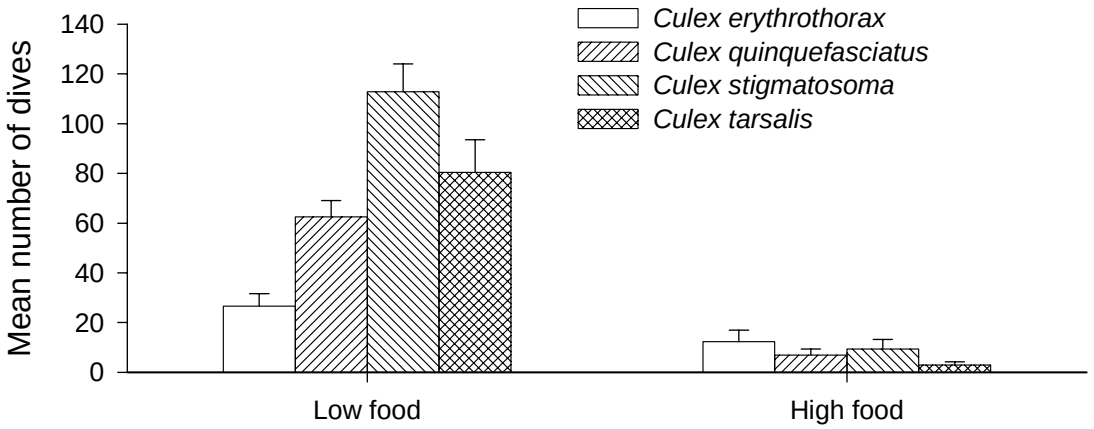

Figure 1. Dive frequencies (all dives: mean ± S.E.) during 15-minute taping sessions of fourth instars of four *Culex* species in low and high food conditions.

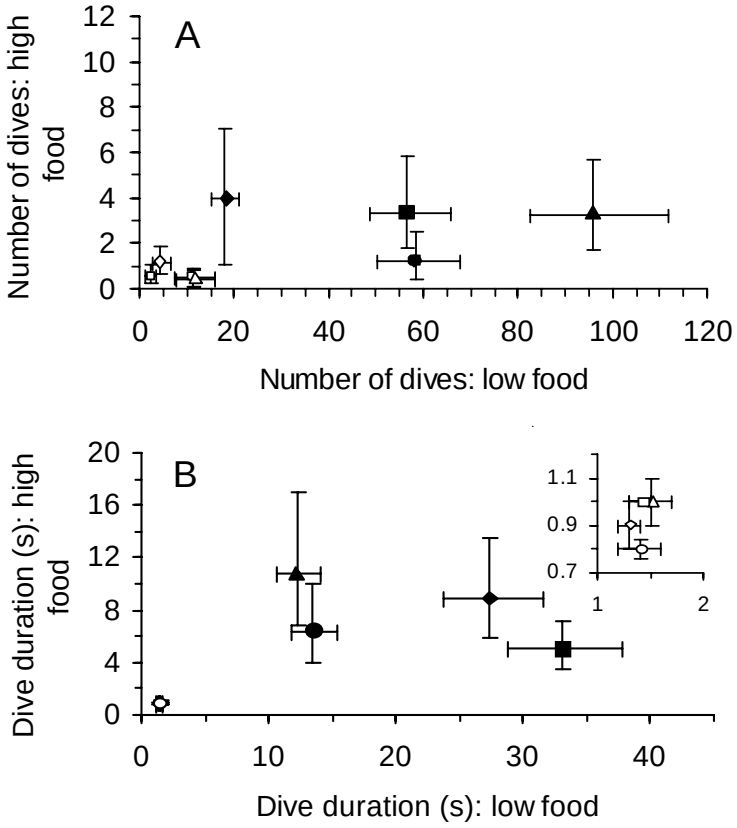

Figure 2. Number (A) and duration (B) of deep and transitional dives (back-transformed least square means ± SE) of fourth instars of four *Culex* species. Deep dives are closed symbols. Transitional dives are open symbols. The dives in lower left of panel B are shown in the insert in the upper right of the panel. ◆ *Culex erythrothorax.* ■ *Cx. quinquefasciatus.* ▲ *Cx. stigmatosoma.* ● *Cx. tarsalis.*

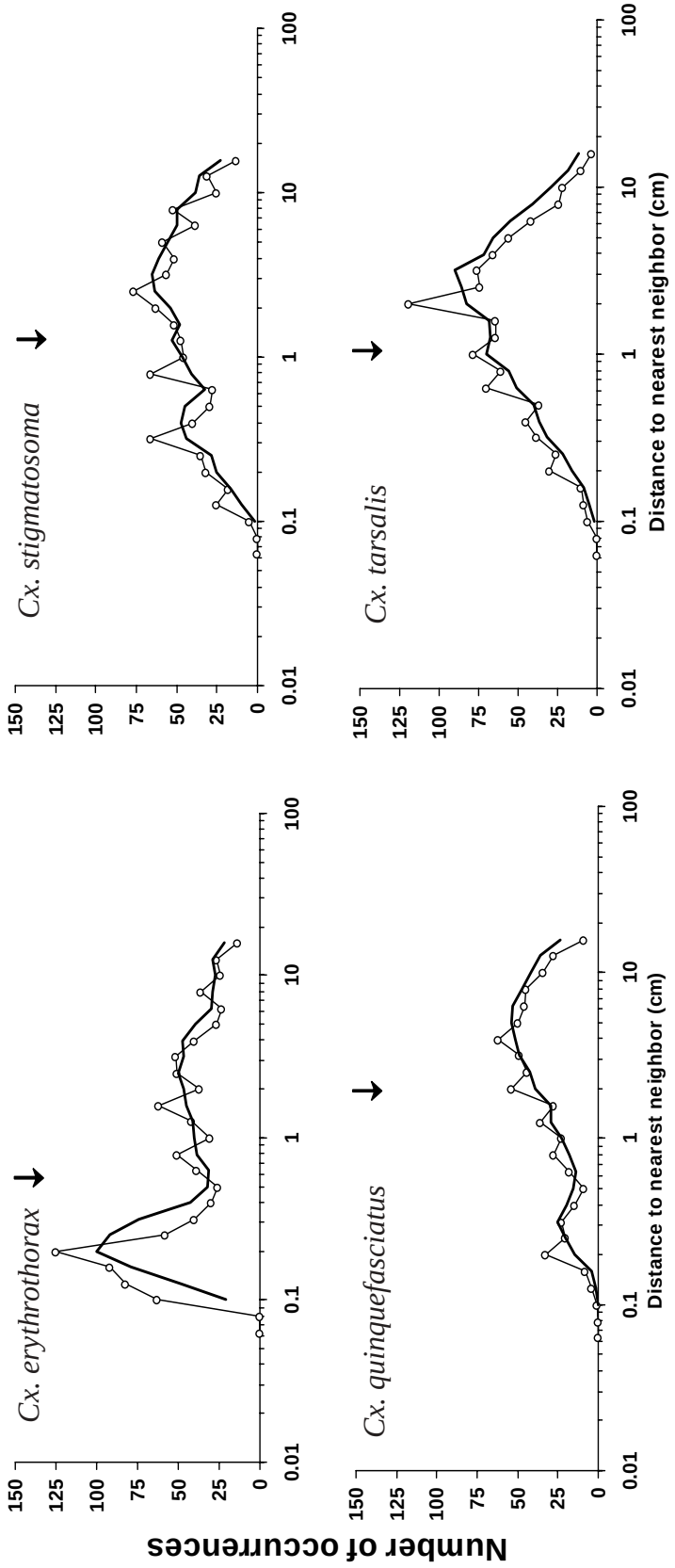

Figure 3. Aggregation of *Culex* larvae in low food environments measured as distance to nearest neighbor. Arrow indicates the back-transformed least square mean for minimum distance between individuals. The bold line represents a running average of 3 points.

Table 2. Comparison of principal components (mean factor score ± SE) among four *Culex* species.

Species	Principal Component		
	1	2	3
Cx. erythrothorax	1.400 ± 0.198[c]	-0.359 ± 0.452[a]	-0.652 ± 0.314[a]
Cx. quinquefasciatus	0.202 ± 0.256[b]	0.399 ± 0.384[a]	1.291 ± 0.263[b]
Cx. stigmatosoma	-0.831 ± 0.071[a]	-0.173 ± 0.389[a]	-0.112 ± 0.232[a]
Cx. tarsalis	-0.571 ± 0.197[a]	0.139 ± 0.243[a]	-0.447 ± 0.216[a]

Means followed by the same letter were not significantly different among species within each column (Bonferroni's method, $P > 0.05$).

contrast, $P<0.05$). Food level did not affect the number of transitional dives executed by *Cx. erythrothorax* and *Cx. quinquefasciatus*.

Dive times

The duration of deep dives (Figure 2B) differed significantly among species ($F_{3,15} = 5.79$, $P < 0.008$) and between food conditions ($F_{1,15} = 14.96$, $P<0.002$; species × food level: $F_{3,15} = 4.26$, $P<0.023$). In high food conditions, deep dive times did not vary significantly among species (contrasts: $P>0.05$). The duration of deep dives in low food conditions differed significantly among the four *Culex* species (contrasts: *quinquefasciatus* = *erythrothorax* > *stigmatosoma* = *tarsalis*). Deep dives under low food conditions were significantly longer than under high food conditions.

The duration of transitional dives in either low or high food conditions (Figure 2) did not vary significantly among the four *Culex* species ($H_3 < 4.38$, $P>0.22$).

Aggregation

The mean for minimum distance between larvae (log-transformed) differed between food levels ($F_{1,27} = 44.86$, $P<0.0005$) and among species ($F_{3,27} = 6.44$, $P<0.002$). The four species responded differently across food levels (species × food level: $F_{3,27} = 4.99$, $P<0.007$). In low food conditions, *Cx. erythrothorax* was the most highly aggregated and differed significantly from the other species (contrast, $P<0.05$; mean minimum distance between larvae [mmd: back-transformed] = 0.61 cm; Figure 3). The least aggregated species in low food conditions was *Cx. quinquefasciatus* (mmd = 1.98 cm). *Culex quinquefasciatus*, *Cx. stigmatosoma* (mmd: 1.38 cm), and *Cx. tarsalis* (mmd: 1.31 cm) did not differ significantly in their degree of aggregation (contrasts, $P>0.05$).

In high food conditions, both *Cx. erythrothorax* (mmd = 0.35 cm, Figure 4) and *Cx. quinquefasciatus* (mmd = 0.36 cm) were more highly aggregated than were *Cx. stigmatosoma* (mmd = 0.79 cm) and *Cx. tarsalis* (mmd = 0.69 cm). The change in the mean distance between larvae across food levels was consistent among three species; *Cx. quinquefaciatus* larvae were more aggregated under high food conditions than expected given their degree of aggregation under low food conditions and the responses of the other species to increased food abundance.

Horizontal distribution of larvae

Under both low and high food conditions, *Cx. erythrothorax* was distributed non-randomly in the aquarium (Figure 5). *Culex erythrothorax* occurred near the corners of the aquarium in both low and high food conditions (54-57% of observations), significantly more frequently than the 10% expectation if larvae were randomly distributed ($\chi^2_3 = 44.36$, $P<0.001$, and $\chi^2_3 = 49.39$, $P<0.001$, low food and high food, respectively). In high food conditions, *Cx. quinquefasciatus* acted in a similar manner, occurring near the corners 38% of the time ($c^2_3 = 35.24$, $P<0.001$). However, *Cx. quinquefasciatus* in low food conditions, as well as *Cx. stigmatosoma* and *Cx. tarsalis* in both low and high food conditions, had horizontal distributions in the aquarium that did not differ significantly from the expectations of random distribution ($\chi^2_3 \leq 1.6$, $P>0.05$, in all cases).

The proportion (arcsine-transformed) of larvae residing in the corners of the aquarium differed significantly among species ($F_{3,27} = 14.89$, $P<0.0005$). The response of *Cx. quinquefasciatus* across food conditions (back-transformed mean proportion: 0.16, low food; 0.38, high food) differed from the responses of the other species (species × food level: $F_{3,27} = 3.72$, $P<0.023$). The proportion of larvae in the corners of the observation chamber did not change significantly across food levels for the three latter species (back-transformed means: *Cx. erythrothorax*: 0.58 (low food) and 0.59 (high

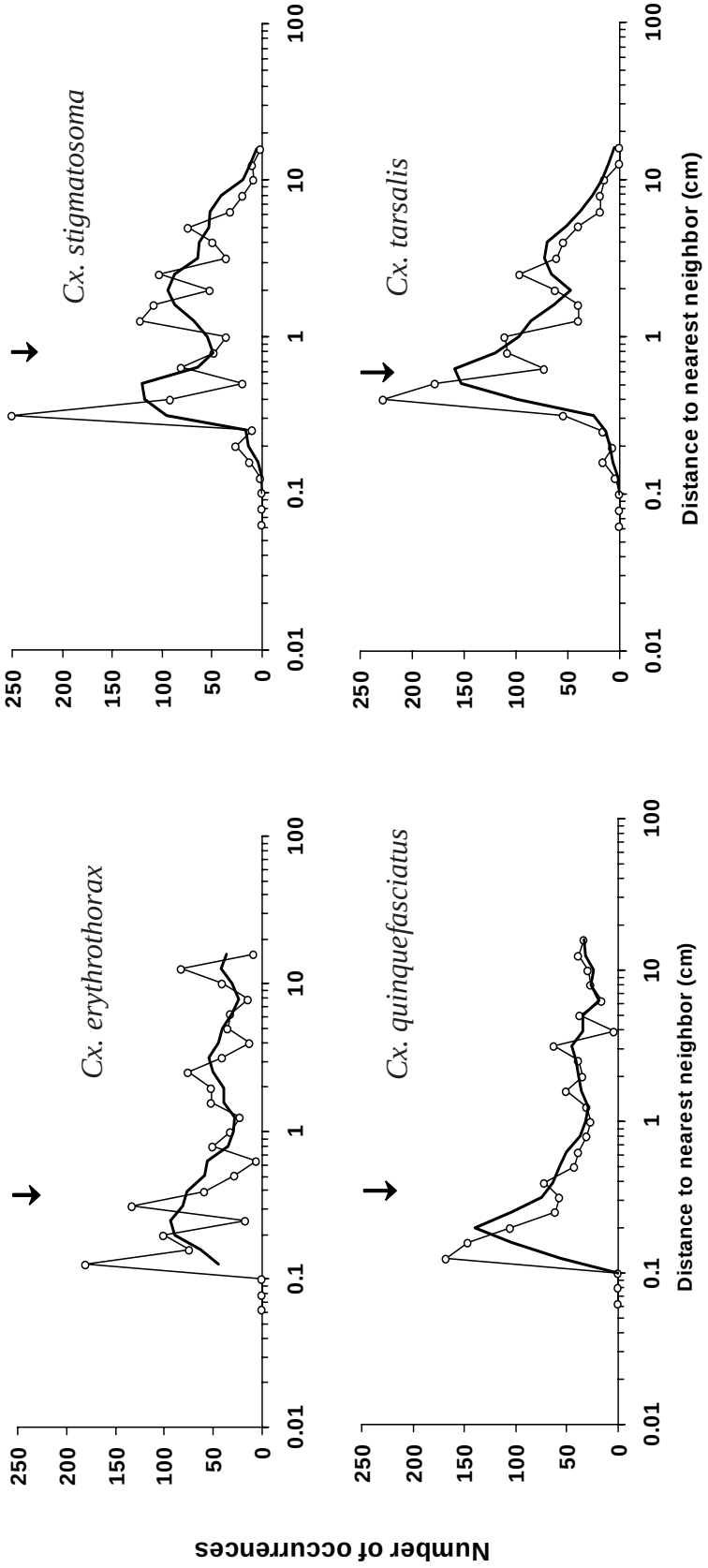

Figure 4. Aggregation of *Culex* larvae in high food environments measured as distance to nearest neighbor. Arrow indicates the back-transformed least square mean for minimum distance between individuals. The bold line represents a running average 3 points.

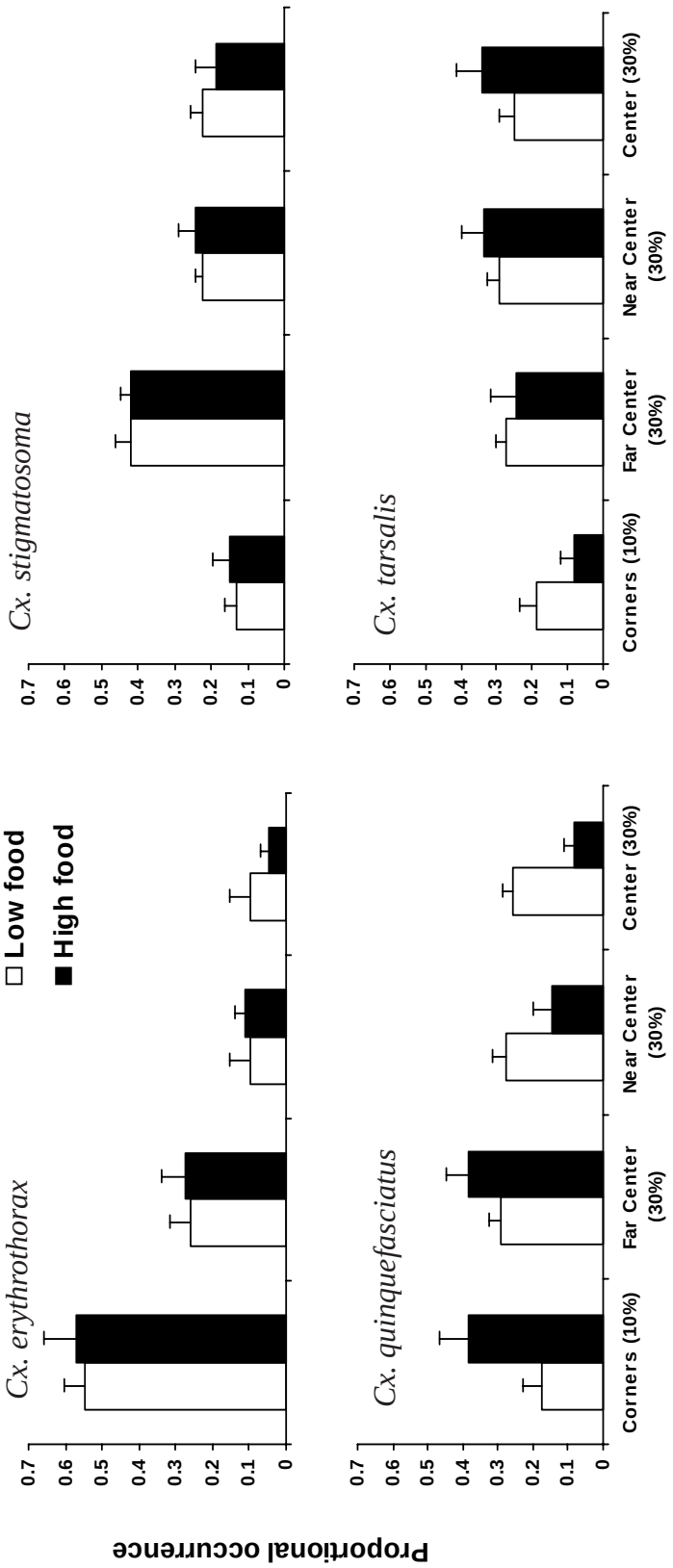

Figure 5. Distribution of *Culex* larvae (mean ± S.E.) in four horizontal zones of the aquarium in both low and high food conditions.

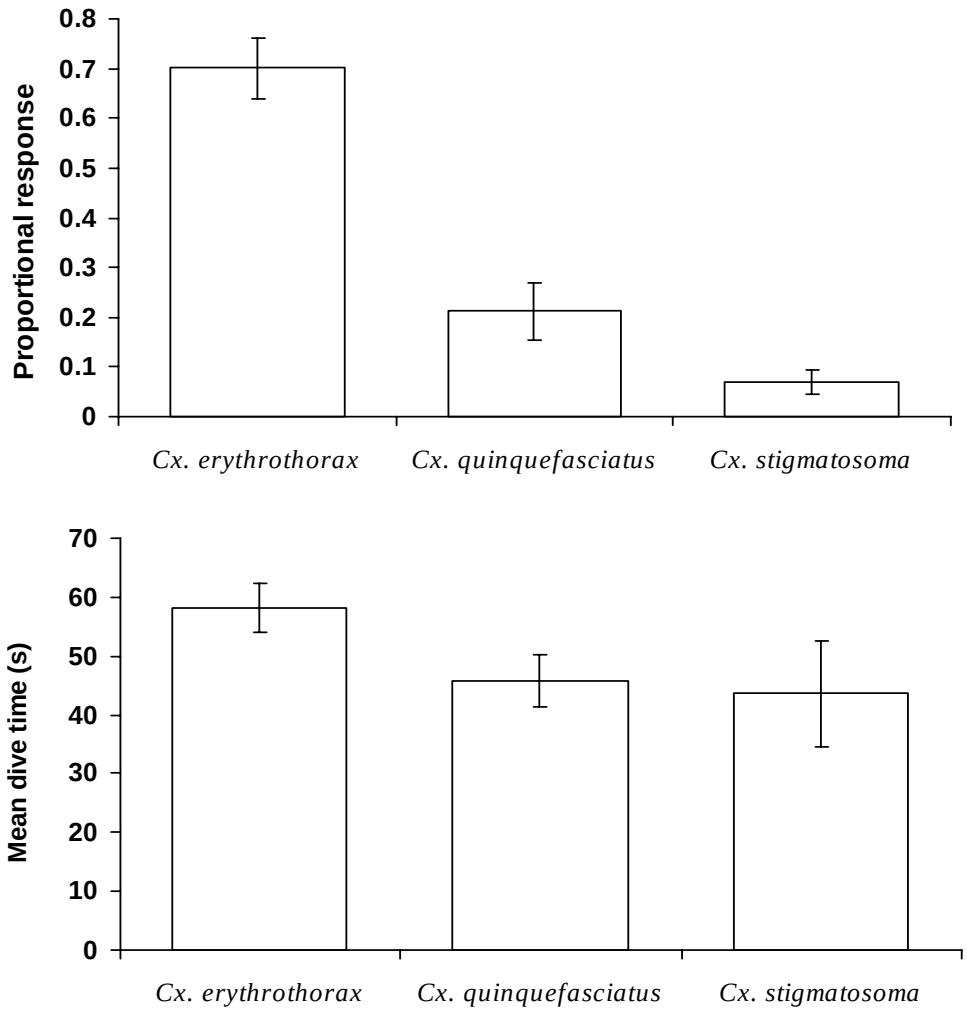

Figure 6. Proportion of larvae responding to a 11% reduction in ambient light level (A) and dive times (mean ± S.E.) for species that responded to a light stimulus (B).

food); *Cx. stigmatosoma* and *Cx. tarsalis*: <0.16); however, a significantly greater proportion of *Cx. erythrothorax* resided in the corners of the observation chamber irrespective of food level (contrasts: low food: *erythrothorax* > *quinquefasciatus*, *stigmatosoma*, *tarsalis*; high food: *erythrothorax* > *quinquefasciatus* > *stigmatosoma*, *tarsalis*).

Response to light stimulus

Culex erythrothorax was most responsive to the light stimulus (Figure 6A), responding significantly more than any of the other three *Culex* species (H_3 = 16.105, P<0.001, Dunn's method, P<0.05). *Culex tarsalis* larvae did not respond to the 11% reduction in ambient light levels. A small percentage of *Cx. quinquefasciatus* and *Cx. stigmatosoma* larvae responded to the light stimulus. No attenuation of the response to the light stimulus was observed for any of the *Culex* species that responded to the light stimulus (χ^2_4 < 3.06, P>0.05).

Dive times for those species that responded to the change in light level did not differ significantly ($F_{2,15}$ = 1.358, P > 0.28; Figure 6B). However, comparison between the dive times of deep dives in low food conditions and dive times elicited by changing light levels showed that dives in response to the external light stimulus were significantly longer ($F_{1,15}$ = 19.45, P<0.001). When responding to a light stimulus, dive times increased 1.3-3.4-fold for *Cx. erythrothorax*, *Cx. quinquefasciatus*, and *Cx. stigmatosoma* and were 32.0, 10.5, and 24.0 s longer, respectively, than were deep dives under low food conditions (*cf.* Figure 2B, Low food and Figure 6B).

Principal component analysis

The three principal components explained 78% of the variance in the behavioral data. Sessions in which larvae responded to a change in light level and were comparatively aggregated in the corners of the aquarium loaded highly on principal component (PC) 1. PC1 explained 42% of the variance in larval behavior and separated behaviors associated with frequent foraging dives from behaviors that indicated a preference for low light conditions (Figure 7). PC2 explained an additional 21% of the variance and separated larval behaviors that changed in relation to food level. Reduced diving activity under high food conditions loaded highly on this axis. Behaviors associated with long dives and comparatively random dispersion of larvae under low food conditions loaded highly on PC3. PC3 explained an additional 15% of the variance in larval behavior.

The factor scores for composite larval behaviors differed among the four mosquito species (Table 2; Pillai's Trace = 1.410; $F_{9,78}$ = 7.68, P<0.001). The mean score on PC1 for *Cx. erythrothorax* and *Cx. quinquefasciatus* differed significantly from each other and from *Cx. stigmatosoma* and *Cx. tarsalis* ($F_{3,26}$ = 27.93, P<0.001; Bonferonni test, P<0.05). The 95% confidence ellipses for *Cx. erythrothorax* and *Cx. quinquefasciatus* behaviors did not overlap with the other species for PC3 vs. PC1 (Figure 7). The factor scores on PC2 did not differ significantly among species ($F_{3,26}$ = 0.79, P>0.5); diving activity under high food conditions did not differ appreciably among the four *Culex* species. *Culex quinquefasciatus* larval behavior on PC3 (least square mean = 1.291) differed significantly ($F_{3,26}$ = 11.18, P<0.001; Bonferonni test, P<0.05) from the other species.

DISCUSSION

The diving behavior, aggregation, and horizontal distribution of fourth instar *Cx. erythrothorax* differed markedly from *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis* larvae, three congeners that co-occur in constructed treatment wetlands in the southwestern United States. Diving activity declined and species-specific differences in behavior were lessened when the four *Culex* species were exposed to high food conditions; yet, the aggregation behavior of *Cx. erythrothorax* larvae and propensity to reside at the corners of the observation chamber changed comparatively little when food level was increased. *Culex erythrothorax* also was sensitive to a small change (89 lm/m^2; 11% reduction) in ambient light level. Though *Cx. quinquefasciatus* and *Cx. stigmatosoma* also responded to a small change laboratory light levels, they were significantly less responsive than *Cx. erythrothorax* larvae. *Culex tarsalis* fourth instars did not respond to the light stimulus.

The differences in larval behavior among the four *Culex* observed in the laboratory have a strong relationship to larval habits in nature. *Culex erythrothorax* larvae were observed to orient their head capsules toward the walls of the aquarium, often brushing the walls with their mouthparts; whereas the larvae of the other species often executed deep dives in search of nutritional resources at the bottom of the aquarium. The behavior of *Cx. erythrothorax* under low food conditions suggests that larvae utilize a scraping-type feeding mode concentrating on vertical structures in a manner similar to *Ochlerotatus atropalpus* Coq. when scraping periphyton from mineral surfaces (Merritt et al. 1992). *Culex erythrothorax* larvae aggregated in the corners of the laboratory aquarium more frequently than did congeners and, in nature, are found primarily in dense stands of vegetation. The number of emerging adults was significantly associated with macrophyte density (Workman and Walton 2000).

Culex quinquefasciatus and *Cx. stigmatosoma* are often found in highly polluted water with little emergent vegetation, such as oxidation ponds and dairy wastewater ponds (Bohart and Washino 1978). The number of *Cx. quinquefasciatus* emerging was not associated with emergent vegetation density in a constructed treatment wetland (Workman and Walton 2000).

Culex tarsalis is not as tolerant of polluted waters as are *Cx. quinquefasciatus* and *Cx. stigmatosoma* but can be found in a broad range of habitat types, particularly habitats associated with agriculture (Bohart and Washino 1978) and containing emergent vegetation (grasses: Walton et al. 1990; macrophytes: Walton and Workman 1998). In constructed treatment wetlands, *Cx. tarsalis* larvae were collected within emergent vegetation and in small areas (~ 1 m^2) of open water within vegetation stands (Workman and Walton 2000, Thullen et al. 2002). The number of emerging *Cx. tarsalis* was not significantly associated with the density of emergent macrophytes underneath emergence traps (Workman and Walton 2000).

Differences of larval behavior among species under low food conditions were greater than under high food conditions. Larval diving under high food conditions decreased 7- to 11-fold relative to low food conditions for three species. *Culex erythrothorax* larvae in high food conditions also dived less than under low food conditions, but carried out fewer dives than did larvae of the other *Culex* under low food conditions. The number of each dive type per session did not vary significantly among the four *Culex* species under high food conditions. As

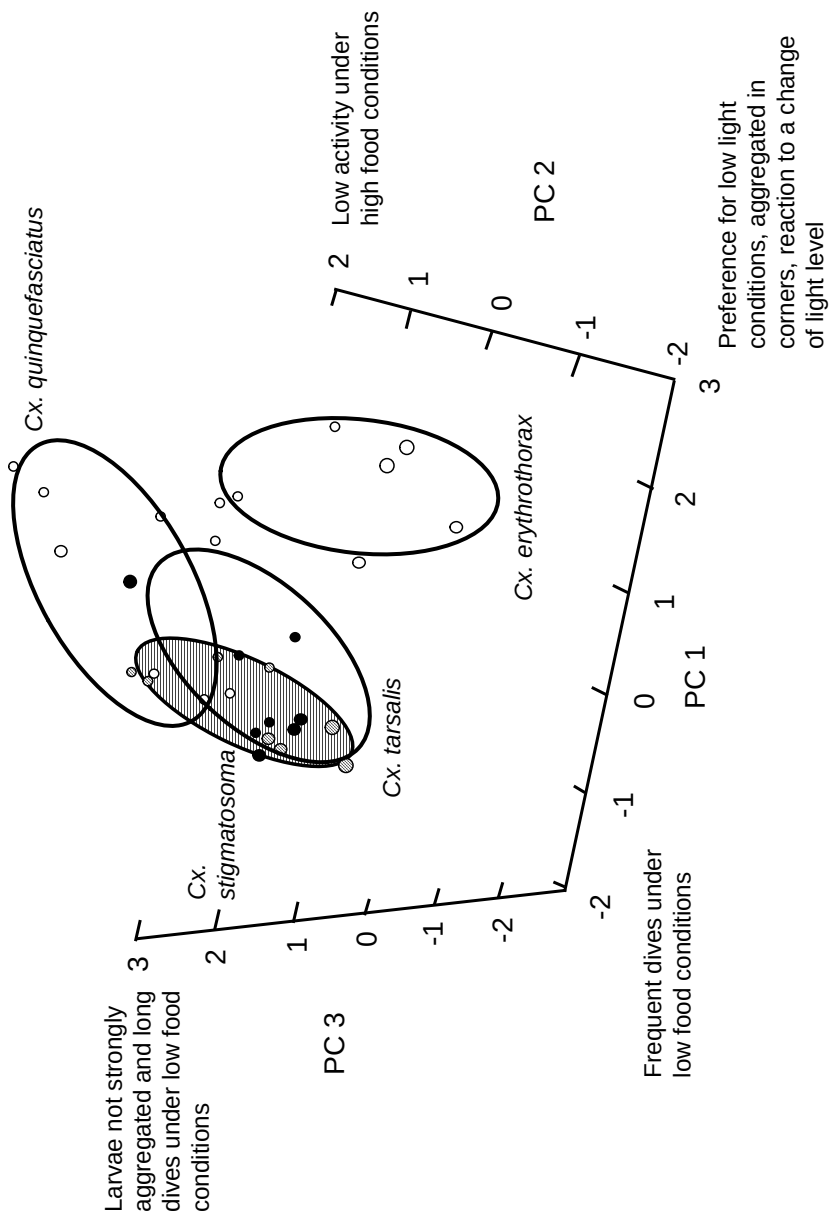

Figure 7. Principal components analysis of behaviors of fourth instars of four *Culex* species. The 95% confidence ellipses are shown for each species. The ellipse and points for *Culex stigmatosoma* are filled with cross hatching. ● are points for *Culex tarsalis*.

was observed under low food conditions, *Cx. erythrothorax* larvae tended to distribute themselves at the periphery of the aquarium. Larvae of all species were observed to feed actively via a collecting-filtering feeding mode and occasionally ejected food boluses. Behaviors of the four southwestern U.S. *Culex* under low food conditions were in rough agreement with the biosystematic generalizations (i.e., diving path, frequency, and position in the water column) for behavior of neotropical *Culex* larvae (Strickman 1989); however, the decreased diving activity and a tendency to reside at the water surface under high food conditions demonstrate that larval behaviors of the four *Culex* species can change significantly with changing resource abundance.

Food levels differentially affected the horizontal distribution of *Cx. quinquefasciatus* larvae. *Culex quinquefasciatus* larvae occurred in the corners of the aquarium under high food conditions but were randomly distributed at the water surface under low food conditions. It is unlikely that this species has a strong preference for vertical structures, therefore under high food conditions in the laboratory, approximately 40% of larvae were often in the corners of the aquarium at the outset of the videotaping sessions and did not move from these locations during the taping.

Feeding behaviors often dominate larval mosquito activity patterns (Walker and Merritt 1991, Juliano and Reminger 1992) and hunger levels also affected the activity patterns of mosquitoes found in non-wetland habitats. The risk of predation by *Toxorhynchites rutilus* Coq. increased for larvae of the tree hole mosquito, *Aedes triseriatus* (Say), as hunger level increased (Juliano et al. 1993). Hungry *Ae. triseriatus* larvae rested less, browsed more frequently, but wriggled (thrashing: Juliano et al. 1993) less frequently than well-fed larvae.

Culex erythrothorax larvae exhibited behaviors indicative of a sensitivity to light, such as aggregation in corners where light levels were low and responsiveness to small changes in ambient light levels. The corners of the observation chamber used in this study were determined to have light levels approximately 8% lower than the center of the observation chamber. The comparatively greater sensitivity of *Cx. erythrothorax* to a small change in ambient lighting has implications for collecting larvae in the field because standard larval sampling methods may generate a disturbance sufficient to cause larvae to dive.

For the three species responding to the light stimulus, the length of dives elicited by a change in light levels was significantly longer than for natural, searching dives. There was also no measurable acclimation of any of the *Culex* larvae that responded to five successive stimuli. Thomas (1950) found that *Cx. fatigans* Wiedemann required at least ten stimulation episodes one min apart before attenuation of responses occurred.

Mosquito larvae are generally very sensitive to changes of light levels and this is probably a defensive behavior (Folger 1946, Thomas 1950, Mellanby 1958). Specifically, larvae dive during a light to dark transition (Leftwich 1954) as was provided by the light stimulus in our study. A response to a rapid decrease of light levels can be a useful defensive behavior for the immature stages of mosquito species that reside in well-lit open water of constructed treatment wetlands, such as *Cx. stigmatosoma* and *Cx. quinquefasciatus*. Both rapid decreases and increases in light levels are likely to signal an impending threat to mosquito larvae dwelling in dimly lit emergent vegetation.

Carrying out escape responses to changes in light levels alone may not be a useful strategy for mosquito larvae, but coupling phototactic responses with a change in visual angle of an object (Hocking 1953) or with a response to mechanical stimuli (Sih 1986) may provide a more reliable indication of a potential threat. The stemmata of mosquito larvae function principally in phototactic responses, and the developing compound eyes in fourth instars are probably sensitive to light (Clements 1999) but are unlikely to form well-defined images. Nevertheless, *Cx. erythrothorax* fourth instars were the most sensitive to small changes in ambient light levels and, while we did not quantify the response of larvae to physical disturbance of the water surface, casual observations indicated that similar species-specific differences in sensitivity to mechanical stimuli were likely.

Observations in this study indicate that *Cx. erythrothorax* has very different larval habits than do three congeners that co-occur in constructed treatment wetlands of the southwestern U.S. The differences in larval behavior observed in this study and the spatial distribution of emerging adult mosquitoes (Workman and Walton 2000) are consonant with *Cx. erythrothorax* larvae spending the majority of their larval development in dense vegetation. Emergent vegetation contributes vertical structure to the water column, may provide supplemental nutritional resources to the food particles suspended in the water column, protects larvae from predators (e.g., notonectids, fish: Orr and Resh 1989, Walton et al. 1990, Walton and Workman 1998) as well as creates other environmental conditions (e.g., temperature, shelter from wind and waves) that enhance larval survivorship (Orr and Resh 1992).

The sensitivity of *Cx. erythrothorax* larvae to changes in light intensity as well as the high degree of aggregation at vertical surfaces may contribute to the difficulty of sampling *Cx. erythrothorax* larvae in nature.

Larval behavior may be an important factor contributing to dipper samples underestimating *Cx. erythrothorax* abundance and the discrepancy in the relative abundance of species for larval versus adult surveys at wetland environments noted in previous studies (Walton and Workman 1998, Workman and Walton 2000).

Acknowledgments

We thank L. Randall for assistance in the field during this project. M. R. Sanford assisted with the determinations of particle density. We appreciate the cooperation of the Eastern Municipal Water District. We benefited from the thoughtful suggestions and comments of J. Keiper, M. Mulla, B. Mullens, T. Payne, P. Walton, M. Wirth and two anonymous reviewers. This research was supported by Special Funds for Mosquito Research from the Division of Agriculture and Natural Resources of the University of California and was carried out as partial fulfillment of an M.S. degree from the Department of Entomology, U.C. Riverside to PDW.

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