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COMPARATIVE EFFICACY OF THE THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS*) AND THE MOSQUITOFISH (*GAMBUSIA AFFINIS*) FOR MOSQUITO CONTROL

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ABSTRACT. The effectiveness of the threespine stickleback as a mosquito control agent was compared to that of the mosquitofish in 28-m^2 earthen ponds during 2 6-wk experiments where the 2 fish were stocked alone and together. Relative to ponds without fish, the stickleback was not effective for controlling larval mosquito populations; however, sticklebacks reduced the abundance of *Culex* pupae. Mosquitofish provided significant levels of control whether stocked alone or concurrently with the stickleback. As compared to mosquitofish alone, mosquito control was not significantly enhanced when both fish were stocked together. Mortality of adult sticklebacks was related to a gradient of increasing water temperature across the ponds rather than the direct effects of other abiotic factors such as low dissolved oxygen concentrations or biotic interactions with the mosquitofish. The stickleback exhibited a lower thermal tolerance and slower population recruitment as compared to the mosquitofish populations, which reproduced successfully in water >33°C and grew rapidly. Stickleback biomass either declined or increased slightly (~50% of initial stocking weight). Mosquitofish biomass increased 33- to 38-fold at rates averaging between 0.079 and 0.095 g wet weight/g/day and total wet weight per pond at 6 wk after stocking did not differ significantly between the 2 mosquitofish treatments.

KEY WORDS *Culex*, larvivorous fish, biological control, mosquitofish, stickleback

INTRODUCTION

The mosquitofish (*Gambusia affinis* Baird and Girard) is the most widely distributed larvivorous fish used for mosquito control (Meisch 1985). The effectiveness of *G. affinis* as a mosquito control agent depends on the abundance of vegetation, the abundance of mosquitoes and relative abundance of other prey, the abundance of mosquitofish predators, and biotic factors affecting mosquitofish reproduction such as fecundity and seasonal breeding cycles (Sawara 1974, Gratz et al. 1996). *Gambusia* has a broad diet that includes phytoplankton, zooplankton, aquatic insects, gastropods, terrestrial insects trapped on the water surface, and eggs and young of fish (Washino and Hokama 1967, Laird 1977, Farley 1980, Hurlbert and Mulla 1981, Miura et al. 1984, Bay 1985). Although mosquitofish reproduction varies seasonally (Sawara 1974, Cech and Linden 1987), maximum recruitment often coincides with peaks in host-seeking adult mosquito activity throughout much of California and the fish's geographic range. Not surprisingly, mosquitofish perform best as a control agent for mosquitoes in situations where vegetation, alternative food sources to mosquitoes, and predatory fish are limited.

The use of mosquitofish has become controversial in recent years because *Gambusia* is purported to affect biodiversity and the abundance of local fauna and, furthermore, the mosquitofish is an effective biological control agent for mosquitoes in a subset of habitats to which the fish has been purposefully introduced (Gamradt and Kats 1996, Gratz et al. 1996, Rupp 1996). This debate has come to the forefront in southern California and in other arid regions where multipurpose constructed wetlands are used for water reclamation, recreation, and wildlife habitat for the maintenance of local and regional biodiversity. Because treatment wetlands can support populations of pestiferous and disease-vectoring mosquitoes and are frequently within proximity to human development, intervention to control mosquitoes is often necessary (Mortenson 1982, Carlson et al. 1986, Walton et al. 1998). In order to fulfill the objectives of maintaining local biodiversity and minimizing mosquito production in constructed treatment wetlands, an effective alternative larvivorous fish to the mosquitofish is needed.

The threespine stickleback (*Gasterosteus aculeatus* L.) has been suggested as an alternative biological control agent for mosquitoes because of its food preferences, behavioral characteristics, and wide geographic distribution (Hubbs 1919, Walton et al. 1996). The threespine stickleback is an endemic species that is distributed throughout California, inhabiting streams, rivers, lakes, and brackish waters (Swift et al. 1993). The stickleback is predaceous and feeds on small organisms throughout the water column (Schooley and Page 1984). Studies have shown that the threespine stickleback has a feeding preference for mosquito larvae and pupae over various other food items (Bay 1985). It has been speculated that simultaneously stocking the top-feeding mosquitofish with a 2nd fish, such as the stickleback, that feeds throughout the water column will provide a level of mosquito control greater than will stocking either fish alone (Woodridge and Davidson 1996).

In this study, the efficacy of the threespine stick-

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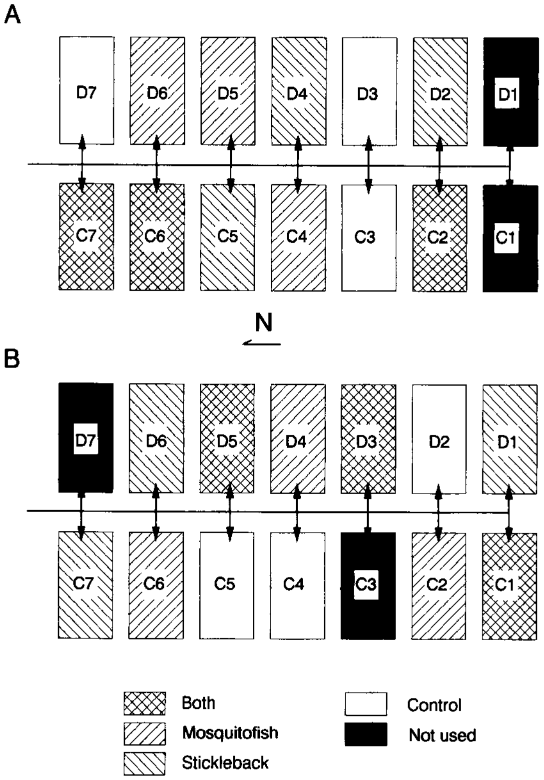

Fig. 1. The arrangement for treatments assigned to ponds at the University of California–Riverside Aquatic Research Facility, Riverside, CA, during 2 studies: (A) 1996 and (B) 1997. Water flow is represented by arrows.

leback as a mosquito control agent was compared to that of the mosquitofish and naturally occurring macroinvertebrate predators in ponds without fish. The potential for the coexistence of the 2 species and the effectiveness of concurrent stocking of both fish as mosquito control agents were also examined.

MATERIALS AND METHODS

Study site: Two experiments were carried out in 12 mesocosms (4 m × 7 m) at the University of California–Riverside (UCR) Aquatic Research Facility. The 1st study was carried out from June 23 to August 12, 1996, and the 2nd study was carried out from April 2 to May 31, 1997. The mesocosms were arranged into 2 rows (rows C and D) (Fig. 1) and water was supplied through a single pipeline from a reservoir at the Agricultural Experiment Station, UCR. All ponds were equipped with float valves that maintained the water depth at approximately 0.3 m. Before the 1996 study, all of the ponds were excavated to approximately 0.16 m, lined with 0.015-mm-thick plastic sheeting, and soil was then replaced over the sheeting.

Upon flooding, each pond was enriched with 3.5 kg of rabbit pellets (Forti-diet, Kaytee Products Inc., Chilton, WI) to promote oviposition by mosquitoes. During the 2nd study, a 2nd enrichment was carried out at 36 days after flooding on May 8, 1997. Five days postflooding (April 7, 1997), all ponds were treated with bifenthrin (Capture® FMC Corporation, Philidelphia, PA) 2EC (20% active ingredient [AI]) at a rate of 0.5 g AI/ha to eliminate populations of predaceous tadpole shrimp (*Triops longicaudatus* Le Conte). This short-lived chemical treatment has no effect on the mosquitoes (Mulla et al. 1992).

Because the mesocosms were devoid of natural vegetation, artificial vegetation was constructed and introduced to each pond before stocking fish. Artificial vegetation consisted of 0.015-mm-thick black plastic sheeting cut into 61 × 5.1-cm strips (Offill 1998[2]). Strips were folded and stapled to form a frond. Five fronds were positioned equidistantly around a 15-cm-diameter circle of 22-gauge galvanized steel wire and weighted with ¾-in. hex nuts. Vegetation was placed in each corner and in the center of each pond to provide visual oviposition cues for mosquitoes and to provide shelter for fish.

Physicochemical measurements: Water temperatures were measured using maximum–minimum recording thermometers (Markson Scientific Inc., Del Mar, CA). The northern ponds in the rows were partially shaded, whereas the southern ponds received full sun. Temperatures were measured in ponds D1, C4, and D6 to provide representative temperature data at various positions within the array of ponds. Thermometers were positioned at approximately 25 cm from the water surface and were read every 48–72 h for the duration of the studies.

The pH, water temperature, and dissolved oxygen concentration were recorded at 30-min intervals over 24 h using electronic sensors (ICM Water Analyzers, Perstorp Analytical, Wilsonville, OR) during the final week of the 2nd study. Measurements were taken at a depth of 18 cm in ponds D1 and D6 on May 27–28, 1997. In order to determine the effect of organic enrichment on pH and the dissolved oxygen concentration, ponds D1 and D6 were again enriched after the 2nd experiment (June 6–7, 1997). The pH, water temperature and dissolved oxygen concentrations were measured under enrichment conditions at 30-min intervals for a 24-h period.

Mosquitoes and nontarget organisms: Mosquitoes and nontarget organisms were sampled by dipper (350 ml) and by tow net (mesh aperture size = 153 μm). Four dips were collected twice each week in the corners of each pond and combined using a concentrator cup (mesh opening = 200 μm). Duplicate tow net hauls were taken weekly along the

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[2] Offill, Y. A. 1998. Comparison of the effectiveness of the three-spined stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis*) for mosquito control. M.S. thesis. University of California, Riverside, CA.

long axis in each pond. All samples were collected between 0700 and 1100 h. Specimens were preserved in alcohol (final concentration approximately 50%). Insects were counted under a stereodissecting microscope at 25–50× and nonculicine taxa were identified to genera using Merritt and Cummins (1984). Mosquito immature stages were separated into early (stages I and II) and late larval instars (stages III and IV) and pupae. All late-stage mosquitoes were categorized to species using Bohart and Washino (1978).

Treatments were assigned to ponds based on initial mosquito larval densities in pretreatment dip samples. Four treatments were assigned so that the variation in mosquito abundance among treatments was equivalent. The treatments were control (no fish stocked), stickleback only, mosquitofish only, and both fish stocked together. Treatments were replicated 3 times.

Fish: Mosquitofish were supplied by Northwest Mosquito and Vector Control District (Corona, CA) and sticklebacks (*Gasterosteus aculeatus microcephalus* Girard) were collected with D-nets from the Mohave River near Apple Valley, CA. Fish were transported in 32-quart ice chests containing water from the collection site which was continuously aerated by portable air pumps. Upon arrival at the UCR facility, fish were acclimated for approximately 24 h in a pond similar to those used for the study. Fish were held in 3 1.25-m^3 cages (1.22 m long × 1.13 m high × 0.91 m wide) constructed of polyvinyl chloride (PVC) frames covered by fiberglass window screening. The top of each cage was covered with plastic netting (Bird Block®, Easy Gardner Co., Waco, TX) to deter piscivorous birds. Minimal losses (<1%) occurred during transport and no losses occurred during acclimation.

Each mesocosm was stocked at a rate of 2.3 kg/ha (approximately 10–14 fish per pond) for the 1996 experiment and 3.4 (each species alone) or 4.5 kg/ha (both fish: approximately 17–22 fish per pond) for the 1997 study. Stocking occurred at 10 and 15 days postflooding for each of the 2 experiments, respectively. Fish were weighed individually and reproductive individuals were distributed equally among treatments. Any fish succumbing during the 1997 experiment were collected using a dip net and measured for standard length. Standard length was converted to wet weight using weight–length regressions (*G. aculeatus*: Walton et al. 1996; dead *G. affinis* were not observed).

Fish yield was estimated at the end of each experiment by collecting fish from each pond using a seine (0.64-cm mesh opening). Three hauls were taken from each pond and a gross wet weight of each species was measured for each haul. Any fish remaining at 2 days after seining and turning off the water supply were collected by dip net and weighed. The distribution of fish wet weight within each treatment was determined by weighing a representative sample of 100 *G. affinis* from each pond. All *G. aculeatus* individuals were weighed. The number of mosquitofish in each pond was estimated by converting the total wet weight to the number of individuals using weight–length regressions for a representative sample of fish ($n = 168$) from the 1996 study. The number of sticklebacks in each pond was determined by direct count.

Statistical analyses: To test for differences in the abundance of mosquitoes among the treatments, abundance data for dip samples were ln-transformed and analyzed using a repeated measures analysis of variance (ANOVA) (Wilkinson and Coward 1997). Immature mosquitoes were categorized into 3 groups: 2 larval subpopulations (1st- and 2nd-stage larvae, 3rd- and 4th-stage larvae) and pupae. Because the variance for each of 2 subpopulations (older larval instars and pupae) was not homogeneous among the treatments in the 1997 study (e.g., no older larval instars were present in some treatments on particular sampling dates), a nonparametric repeated-measures ANOVA was used to test for differences among the 4 treatments. Pairwise comparisons between treatment means for each of the 3 subpopulations were made using the Student–Newman–Keuls method (Fox et al. 1995). Similar analyses were carried out for nontarget organisms that were sufficiently abundant to permit statistical comparisons among the treatments.

In order to compare fish production among the treatments containing fish, treatment means were calculated for biomass (wet weight) of each species and compared using a *t*-test. The estimated number of mosquitofish per pond was compared between treatments (mosquitofish only vs. both fish stocked together) using a *t*-test.

RESULTS

Physicochemical factors

A north–south gradient in temperature occurred across the ponds. Maximum water temperatures of ponds at the south end of the rows were 2–5°C warmer than were water temperatures in partially shaded ponds at the north end of the rows during the 1996 study (Fig. 2A). In the warmest pond, D1, the average maximum water temperature was 35.3°C and the minimum was 21.1°C. In C4 and D6, the maximum water temperature averaged across sample dates was 33.5°C and the average minimum temperatures were 21.9 and 22.1°C, respectively.

Maximum water temperatures during the 20 days after stocking fish in 1997 (Fig. 2B) were 5–7°C cooler than those recorded during the 1996 study (Fig. 2A) and the 2nd half of the 1997 study. Pond D1 was the warmest, with an average maximum water temperature of 32.5°C and an average minimum temperature of 20.6°C throughout the 1997 study. These averages were only slightly higher

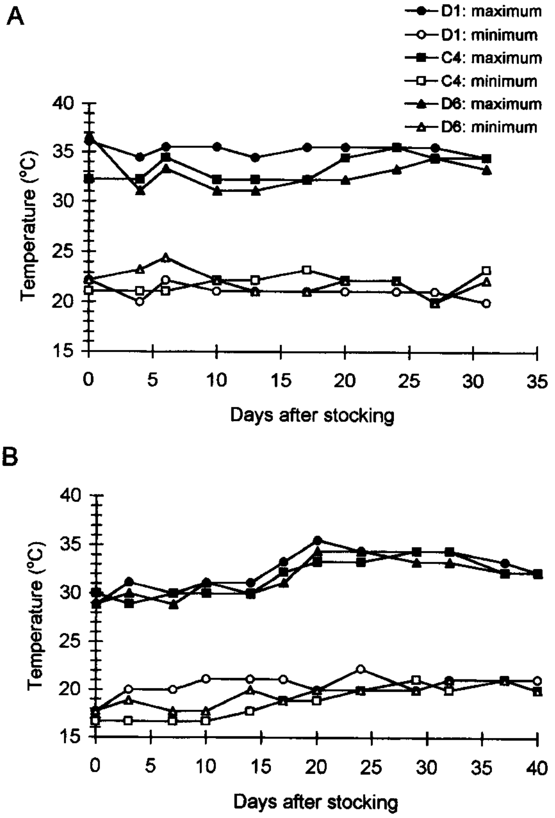

Fig. 2. Maximum and minimum water temperatures in 3 ponds at the University of California–Riverside Aquatic Research Facility, Riverside, CA, (A) from June 23 to August 12, 1996 and (B) from April 2 to May 31, 1997.

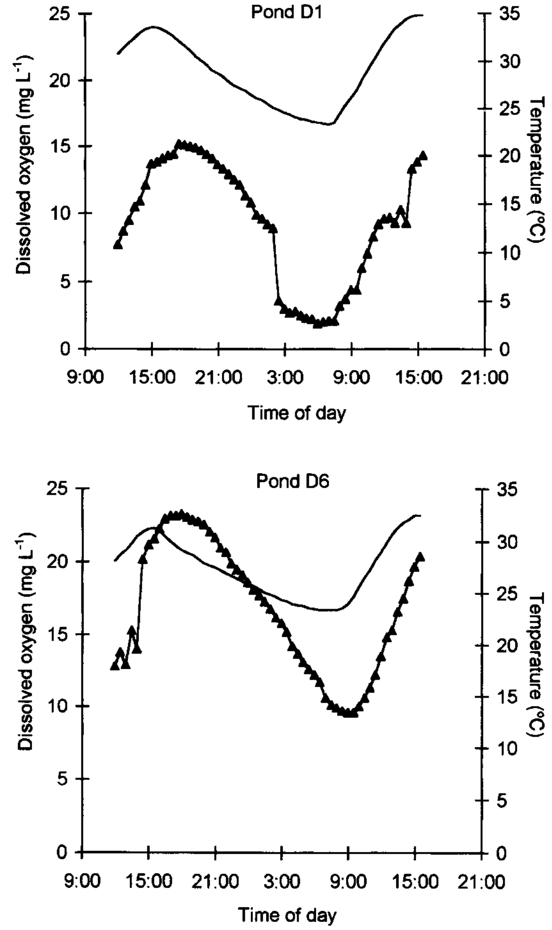

Fig. 3. Dissolved oxygen concentration (▲) and water temperature (—) measured over a 24-h period (May 27–28, 1997) in 2 ponds at the University of California–Riverside Aquatic Research Facility, Riverside, CA, at 3 wk postenrichment.

than those for ponds C4 and D6, which had average maximum temperatures of 31.8 and 31.6°C, respectively, and average minimum temperatures of 18.7 and 19.5°C, respectively.

Dissolved oxygen concentrations at 3 wk after enrichment fluctuated between 11–13 mg/liter during a 24-h period (Fig. 3). The dissolved oxygen concentration in pond D1 dropped to 2 mg/liter during the night and subsequently increased rapidly beginning at 0900 h. The amplitude of the diel fluctuation of dissolved oxygen concentrations in the cooler pond, D6, was similar to that observed in pond D1; however, the minimum dissolved oxygen concentration was approximately 10 mg/liter during late May (Fig. 3). Because the northern ponds were shaded during the morning, the daily increase of dissolved oxygen concentration in pond D6 began approximately 2 h later than in pond D1.

Nighttime dissolved oxygen concentrations decreased appreciably shortly after enrichment compared with dissolved oxygen measurements taken 20 or more days following enrichment. Dissolved oxygen concentration declined abruptly during the late afternoon and early evening (after 1630 h) and was 0 mg/liter throughout the night (Fig. 4). Dissolved oxygen concentration remained at 0 mg/liter for approximately 13 h in pond D6 and for 9 h in pond D1. After enrichment, the maximum dissolved oxygen concentrations in the ponds were 9–10 mg/liter.

Mosquitoes and nontarget organisms

The predominant mosquito species collected during both studies were *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex tarsalis* Coq. During the 1996 study, *Cx. tarsalis* and *Cx. stigmatosoma* were more common than was *Cx. quinquefasciatus* (average relative abundance = 47, 48, and 5%, respectively). *Culex stigmatosoma* and *Cx. tarsalis* were also prevalent during spring 1997; average relative abundance was 37 and 47%, respec-

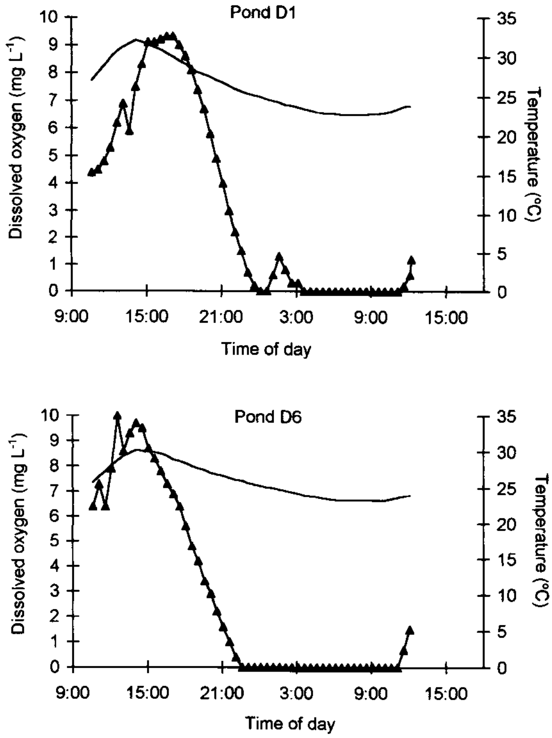

Fig. 4. Dissolved oxygen concentration (▲) and water temperature (—) measured over a 24-h period (June 6–7, 1997) in 2 ponds at the University of California–Riverside Aquatic Research Facility, Riverside, CA, at 2 days after enrichment.

tively. Only 2 *Culiseta* sp. larvae were collected during the 1997 experiment.

In 1996, all sticklebacks died in both treatments. Because of this mortality, the 1996 data were analyzed for the presence versus absence of mosquitofish. Mosquitofish provided significant levels of control for larval (1st and 2nd instars: $F_{1,10} = 6.17$, $P < 0.032$; 3rd and 4th instars: $F_{1,10} = 5.96$, $P < 0.035$) and pupal ($F_{1,8} = 14.01$, $P < 0.006$) mosquito subpopulations as compared to ponds without fish. Late-stage (3rd and 4th instars) mosquito abundance declined from approximately 250 larvae per dip during the 1st week after stocking mosquitofish to less than 3 larvae per dip from 17 to 31 days after stocking fish (Fig. 5). In contrast to the 100-fold reduction in larval abundance observed between days 10 and 17 in ponds containing the mosquitofish, the abundance of late instars in ponds without mosquitofish declined approximately 30-fold between the 1st and 3rd week of the experiment. During the last half of the 1996 study, the late-stage subpopulations in ponds without *Gambusia* were 5- to 10-fold larger than in ponds containing mosquitofish and averaged between 7 and 20 larvae per dip.

Gambusia did not significantly affect the abundance of nontarget organisms (repeated measures ANOVAs, $P > 0.05$). Cyclopoid copepods were the most abundant potential predator of mosquito larvae during the 1996 experiment (Table 1). Predatory insects were rare.

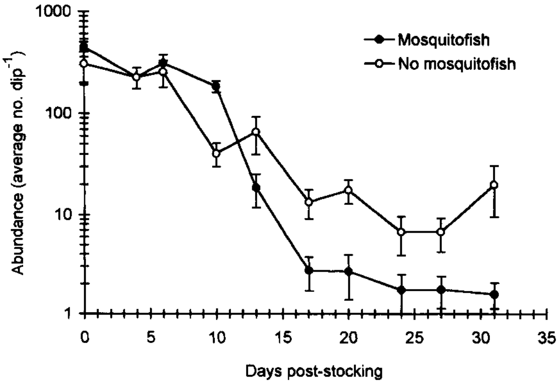

Fig. 5. Abundance (mean ± SE) of *Culex* spp. 3rd- and 4th-stage larvae in dip samples from treatments with and without mosquitofish during the period from June 23 to August 12, 1996.

In 1997, sticklebacks failed to provide significantly better mosquito control than did the naturally occurring invertebrate predators in the control treatment (Table 2). This was true for both larval mosquito subpopulations (Table 2). Many of the sticklebacks survived in the 1997 experiment and, therefore, comparisons of the effects of the 4 treatments on mosquitoes and nontarget organisms were possible. The abundance of both larval subpopula-

Table 1. Nontarget taxa collected from experimental ponds at the University of California–Riverside Aquatic Research Facility in Riverside, CA, from June 23 through August 12, 1996, and from April 2 through May 31, 1997.

	Abundance[1]	
Nontarget group	1996	1997
Anisoptera: Aeshnidae	R	R
Anisoptera: Libellulidae	R	R
Ceratopogonidae	U	R
Chironomidae	C	A
Cladocera	A	A
Copepoda	A	A
Corixidae	R	U
Dytiscid larvae	R	R
Ephemeroptera	U	R
Ephydrid larvae	U	R
Hydrophilid larvae	R	R
Laccophilus spp.	R	R
Notonectidae	R	A
Ostracoda	C	R
Veliidae	R	R
Zygoptera: Coenagrionidae	R	R

[1] A, abundant (≥10,000 individuals collected); C, common (1,000 ≤ C < 10,000 individuals collected); U, uncommon (100 ≤ U < 1,000 individuals collected); R, rare (<100 individuals collected).

Table 2. Pairwise comparisons of larvivorous fish treatment means for the abundance of immature *Culex* spp. collected in dip samples during 1997.

Stage[1]	Comparison	Difference of means or ranks	*n*[2]	*q*[3]	$P < 0.05$
LI–LII	Stickleback vs. mosquitofish	1.99	4	6.70	Yes
	Stickleback vs. both	1.55	3	5.22	Yes
	Stickleback vs. control	0.18	2	0.61	No
	Control vs. mosquitofish	1.81	3	6.09	Yes
	Control vs. both	1.37	2	4.61	Yes
	Both vs. mosquitofish	0.44	2	1.48	No
LIII–LIV	Stickleback vs. mosquitofish	26.0	4	5.59	Yes
	Stickleback vs. both	20.0	3	5.55	Yes
	Stickleback vs. control	4.0	2	1.57	No
	Control vs. mosquitofish	22.0	3	6.10	Yes
	Control vs. both	16.0	2	6.28	Yes
	Both vs. mosquitofish	6.0	2	2.35	No
Pupae	Stickleback vs. mosquitofish	22.0	4	4.73	Yes
	Stickleback vs. both	19.0	3	5.27	Yes
	Stickleback vs. control	1.0	2	0.39	No
	Control vs. mosquitofish	21.0	3	5.82	Yes
	Control vs. both	18.0	2	7.06	Yes
	Both vs. mosquitofish	3.0	2	1.18	No

[1] L, larval instar.

[2] *n*, number of treatments in the comparison.

[3] *q*, Student–Newman–Keuls statistic.

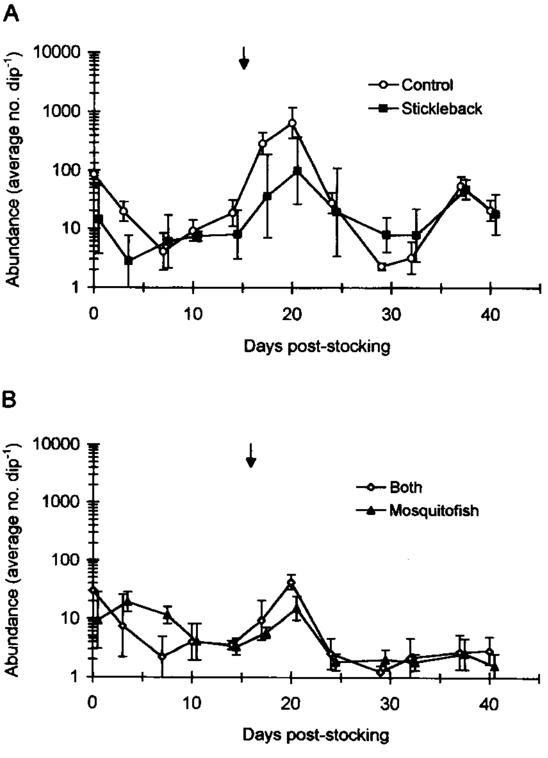

Fig. 6. Abundance (mean ± SE) of *Culex* spp. 1st- and 2nd-stage larvae in dip samples from treatments (A) with stickleback and no fish (control) and (B) with mosquitofish and both mosquitofish and sticklebacks during the period from April 2 to May 31, 1997. The arrow indicates the 2nd enrichment. Points are offset horizontally to facilitate illustration.

tions (1st and 2nd instars: $F_{3,8} = 5.02$, $P < 0.030$; 3rd and 4th instars: $\chi^2_3 = 21.89$, $P < 0.001$) and pupae ($\chi^2_3 = 18.69$, $P < 0.01$) differed significantly among the 4 treatments. Although larval abundance in the stickleback treatment increased 10-fold after the 2nd enrichment (Figs. 6A and 7A), the decline in both larval subpopulations across the experiment was negligible compared to that observed in the mosquitofish treatments (Figs. 6B and 7B). Late instars in ponds without fish declined approximately 10-fold during the 1st week of the experiment and then increased to an average of 200 larvae per dip after the 2nd enrichment of the ponds. Populations of older larvae in the control ponds declined by 2 orders of magnitude between day 20 and 30 (Fig. 7A).

The abundance of *Culex* pupae was greatly reduced in treatments with mosquitofish and reduced to a lesser extent in ponds containing only the stickleback (Table 2). Although the number of pupae present in the stickleback and control ponds fluctuated throughout the study (Fig. 8A), pupal abundance in ponds containing sticklebacks alone declined until day 20 after stocking and resurged thereafter to approximately 10 pupae per dip. The number of pupae present in ponds with mosquitofish or both fish together decreased after the 1st 10 days and remained suppressed throughout the experiment (Fig. 8B).

The mosquitofish provided significantly better control of immature mosquitoes as compared to both the naturally occurring macroinvertebrates in the fishless ponds and the stickleback (Table 2). The treatment with both fish present also showed

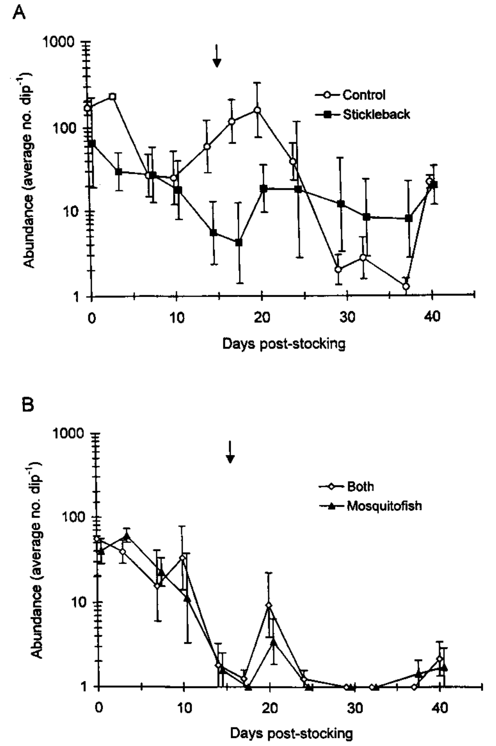

Fig. 7. Abundance (mean ± SE) of *Culex* spp. 3rd- and 4th-stage larvae in dip samples from treatments (A) with stickleback and no fish (control) and (B) with mosquitofish and both mosquitofish and sticklebacks during the period from April 2 to May 31, 1997. The arrow indicates the 2nd enrichment. Points are offset horizontally to facilitate illustration.

significantly improved control for all stages of immature mosquitoes relative to the control and stickleback treatments (Table 2). The abundance of late instars declined from approximately 50 larvae per dip at the time of stocking to nearly undetectable levels after 17 days (Fig. 7B). Population trends for the 2 larval subpopulations were similar in both *Gambusia* treatments. Larval mosquito abundance did not differ significantly between the mosquitofish alone and the mosquitofish + stickleback treatments (Table 2).

The mosquitofish also quickly reduced larval mosquito populations after a 2nd enrichment. Late-stage mosquito abundance was only about 3 and 9 larvae per dip in the mosquitofish and the combined fish treatments, respectively, after the 2nd enrichment of the ponds (Fig. 7B). In ponds containing *Gambusia*, the abundance of late-stage mosquitoes declined to preenrichment levels by the next sampling date. After the 2nd enrichment, late-stage populations in the ponds containing mosquitofish were 15–45% of those in the stickleback treatment (approximately 50 larvae per dip: Figs. 7A, 7B) and 2–5% those in the control treatment (200 larvae per dip: Fig. 7A).

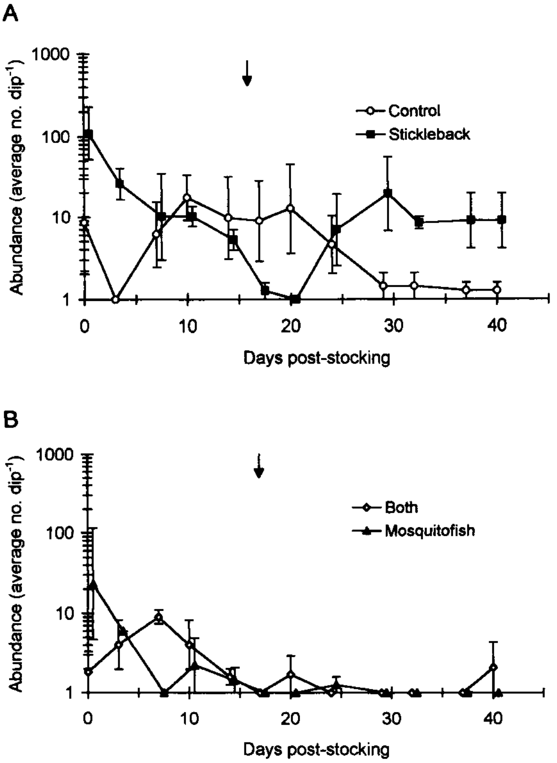

Fig. 8. Abundance (mean ± SE) of *Culex* spp. pupae in dip samples from treatments (A) with stickleback and no fish (control) and (B) with mosquitofish and both mosquitofish and sticklebacks during the period from April 2 to May 31, 1997. The arrow indicates the 2nd enrichment. Points are offset horizontally to facilitate illustration.

No significant differences were found in nontarget species abundance among treatments (repeated measures ANOVAs; $P > 0.05$). Species abundance varied slightly between seasons (Table 1). Copepods and cladocerans were the most abundant zooplankton throughout the experiment. Abundance of notonectids during the 1997 experiment was greater than during 1996.

Fish populations

Although sticklebacks did not survive to the end of the 1996 study, the mosquitofish populations increased substantially. At 6 wk after stocking, mosquitofish biomass (wet weight) per pond increased an average of 21-fold (from 5.80 to 118.6 g) when stocked alone and 44-fold (from 3.5 to 155.5 g) when stocked concurrently with the stickleback (Fig. 9A). Except for 1 pond in the mosquitofish alone treatment where the mosquitofish population increased only about 10-fold, the wet weight of the resultant fish populations in the 2 mosquitofish treatments was similar, approximately 150 g per pond (53.6 kg/ha). The average increase for mosquitofish biomass was approximately 33-fold. The

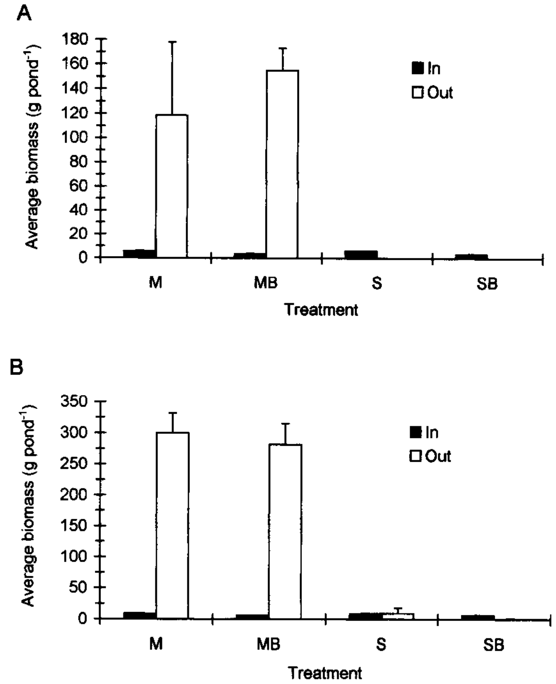

Fig. 9. Fish biomass at stocking (In) and after 6 wk (Out) for 2 studies at the University ofCalifornia–Riverside Aquatic Research Facility, Riverside, CA, (A) summer 1996, (B) spring 1997. M, mosquitofish alone; MB, mosquitofish when both fish species were present; S, stickleback alone; SB, stickleback when both fish species were present.

final biomass of mosquitofish in the 2 treatments did not differ significantly (t_4 = 1.028; P = 0.36).

During the 1997 study, the average stickleback biomass per pond decreased from 9.71 to 9.56 g when stocked alone and from 6.56 to 0.330 g when stocked with the mosquitofish (Fig. 9B). Stickleback mortality occurred 1st (May 1) and was greatest in the comparatively less shaded southern ponds (ponds C1 and D1). On May 11, dead adult sticklebacks were also found in ponds D3, D5, and D6. For the entire study, the number of dead adult sticklebacks collected by dip net declined along the north–south gradient of ponds (pond [number of dead adults collected]): C1 (8), D1 (9), D3 (4), D5 (6), D6 (1), and C7 (1). Despite these losses, the surviving sticklebacks in the 2 northernmost ponds successfully reproduced and between 10 and 20 juveniles per pond were recovered by seining. Stickleback biomass (wet weight) in ponds C7 and D6 increased approximately 50% from that stocked.

Seining removed >98% of the mosquitofish biomass from the earthen ponds, with the exception of pond D5, where only 73% of the fish biomass was removed by seining. The bottom of pond D5 was more irregularly shaped than were the bottoms of the other ponds; a subset of the fish in D5 was able to avoid the seine.

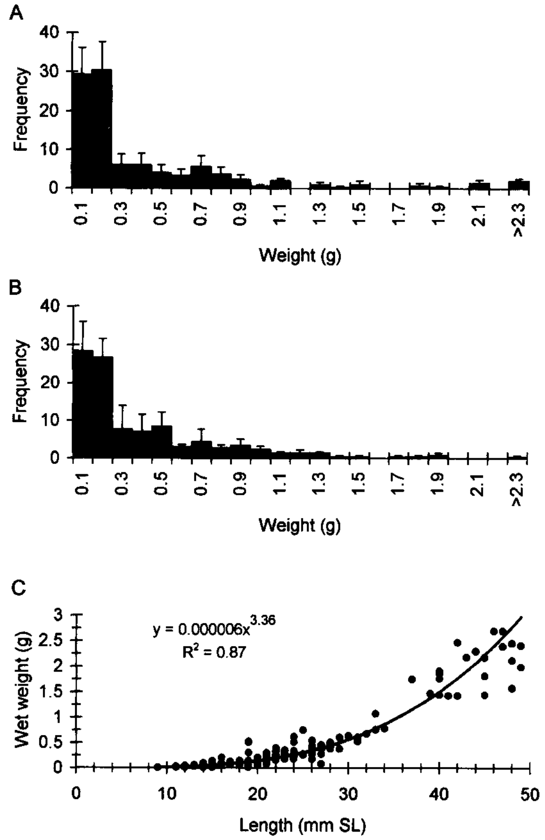

Fig. 10. Relative abundance of *Gambusia affinis* weight (wet weight) classes in the (A) mosquitofish alone and (B) both fish treatments and (C) the relationship between mosquitofish wet weight and standard length in ponds at the University of California–Riverside Aquatic Research Facility, Riverside, CA. The mean ± SD is illustrated for each weight category.

The biomass of the mosquitofish population increased almost 38-fold during the 2nd study and, after 6 wk, was approximately 2-fold larger than during summer 1996. The average mosquitofish biomass per pond increased from 9.23 to 299.96 g in the mosquitofish-only treatment and from 6.08 to 281.94 g in ponds with both fish species (Fig. 9B). The final biomass of mosquitofish in the 2 treatments (mosquitofish alone: 107.13 kg/ha; both fish: 100.7 kg/ha) did not differ significantly (t_4 = 0.964, P = 0.39). The distribution of *G. affinis* among the weight size categories was similar for 2 mosquitofish treatments (Figs. 10A, 10B). The majority of individuals (approximately 60%) were in the 0.1 and 0.2 g weight classes. Wet weight increased directly as a cubic power of length (Fig. 10C).

The estimated number of *G. affinis* per pond was similar in the mosquitofish-only ponds (mean ± SD: 896 ± 96) than in the ponds stocked with both fish species (843 ± 99). The estimated number of

G. affinis per pond did not differ significantly between the 2 mosquitofish treatments ($t_4 = 0.67$, $P < 0.55$).

DISCUSSION

The stickleback was less effective than was the mosquitofish for reducing populations of immature mosquitoes. Even though sticklebacks were not an effective control agent for *Culex* larvae, *G. aculeatus* provided significantly better control of mosquito pupae than did the naturally occurring macroinvertebrate predators in the ponds without fish at 2–3 wk after stocking fish in the 1997 study. Although sticklebacks persisted during the 1997 experiment, the abundance of mosquito larvae in stickleback and control treatments did not differ significantly. This lack of a significant difference for mosquito abundance between the 2 treatments was in part due to the variability in mosquito larval abundance among ponds assigned to the stickleback treatment and was caused by differential mortality of sticklebacks among the ponds. Stickleback mortality was 1st observed at day 20 after stocking and thereafter pupal abundance increased in ponds stocked with only *G. aculeatus.* The significant reduction of the pupal subpopulation by *G. aculeatus* might have been caused by enhanced detection of the darkly pigmented pupae as compared to the more lightly pigmented larvae.

The mortality of the stickleback was caused by thermal stress rather than the direct effects of other abiotic factors such as low dissolved oxygen concentrations or biotic interactions with the mosquitofish. The timing of stickleback mortality during the 1996 experiment was unknown. Maximum water temperatures were ≥31°C throughout the 1996 experiment and, in the warmest ponds at the south ends of the rows, maximum water temperatures were ≥35°C for much of the study. During the summer months, water temperatures reached a maximum of 36.7°C. Because thermometers were situated at approximately 0.25 m depth, the temperature at the water surface might have been slightly higher than those recorded by the thermometers.

An increase in the maximum water temperature to >33°C was associated with death of reproductive sticklebacks during the 2nd study and mortality was directly related to the gradient of water temperature observed in the ponds. Stickleback mortality was observed at around 20 days after stocking in 1997. Before day 20, maximum water temperatures were ≤31°C. After day 20 poststocking, maximum water temperatures were between 33 and 35°C. The daily maximum temperature in the warmest pond of the 1997 study averaged 35.3°C. Water temperatures probably exceeded the thermal tolerance of the stickleback subspecies (*G. aculeatus microcephalus*) used in our study. The thermal tolerance for a related subspecies, *G. aculeatus williamsoni* Girard, collected from the Santa Clara River in Los Angeles County, was approximately 34°C (Feldmeth and Baskin 1976). The geographic ranges of *G. a. microcephalus* and *G. a. williamsoni* overlap in the Los Angeles Basin (Swift et al. 1993). Upper lethal temperatures were even lower (28.8–21.6°C) in tests of thermal and osmotic acclimation for the euryhaline *G. aculeatus aculeatus* (L.) collected from the middle of its eastern geographic range in Halifax, Nova Scotia, Canada (Jordan and Garside 1972).

Even though the stickleback is primarily a midwater fish (Schooley and Page 1984), it can withstand low dissolved oxygen levels by altering behavior and swimming at, or just below, the water surface (Whoriskey et al. 1985, Walton et al. 1998). Under normal conditions in the Riverside ponds, the dissolved oxygen concentration was lowest between approximately 0200 and 0700 h. The nighttime dissolved oxygen concentration decreased to approximately 2 mg/liter in pond D1 and was 5 times greater in a cooler pond, D6. These levels are sufficient for normal swimming behavior of the stickleback (Feldmeth and Baskin 1976). Under enrichment conditions, dissolved oxygen levels in the Riverside ponds were sustained at 0 mg/liter for periods of 9–13 h during the late afternoon through midmorning. Hypoxia caused by the 2nd enrichment might have forced the sticklebacks to reside in thermally stressful conditions near the water surface. Other studies indicated that the stickleback was able to survive periods of oxygen deprivation when water temperature was <29°C (Whoriskey et al. 1985, Walton et al. 1997).

Mortality of sticklebacks stocked concurrently with mosquitofish was no greater than for sticklebacks stocked alone. Even though the mean final biomass of sticklebacks in the 2 treatments differed appreciably in 1997, sticklebacks were eliminated from the warmest ponds in both treatments and, consequently, the variation around mean final biomass for each treatment was large. Although mosquitofish biomass increased appreciably (average increase 38 times initial wet weight) over the course of the experiment, the stickleback biomass increased a comparatively small amount (~50% initial stocking weight) or declined. In this study, mosquitofish and sticklebacks were able to coexist and successfully reproduce in relatively cool ponds. Long-term studies may be more appropriate to evaluate the persistence of *G. aculeatus* because the sticklebacks exhibit complex mating behaviors and require sufficient time for nesting, mating, hatching of eggs, and paternal care of the young before their open-water dispersal. Because of the short duration of this study, an evaluation of the long-term persistence of both species when reared together was not possible. Coykendall (1980) cited observational evidence that resident stickleback populations excluded mosquitofish from several types of aquatic habitats in Oregon.

The mosquitofish, whether stocked alone or with the stickleback, provided significantly better mosquito control than did the stickleback alone or the predaceous aquatic macroinvertebrates in the ponds without fish. The mosquitofish reproduced rapidly. Mosquitofish production is directly related to primary production (Goodyear et al. 1972) and exhibits seasonality (Sawara 1974, Cech and Linden 1987). During 1997, mosquitofish populations grew at rates between 0.651 and 0.671 individuals/individual/wk. Population growth rates were somewhat greater than those observed in longer studies (0.288 individuals/individual/wk: 13- to 14-wk study of Reed and Bryant [1974]; 0.401–0.414 individuals/individual/wk: 10-week study of Hoy and O'Grady [1971]) where resource limitation and seasonal reduction in reproduction might have occurred. The ability to reproduce rapidly following introduction and wide environmental tolerances (Brett 1956) are favorable characteristics of the mosquitofish, particularly in the environmentally stressful conditions found in constructed treatment wetlands (Walton et al. 1997).

No significant difference was found in mosquito control between the treatment with both fish and the mosquitofish alone. The reduction of larval mosquito populations in the treatment with both fish can therefore be attributed to the presence of the mosquitofish. Our data do not support an enhancement of mosquito control by the concurrent stocking of mosquitofish with a fish, such as the stickleback, that feeds throughout the water column. We conclude that mosquito control is not benefited by simultaneously stocking both fish species.

The lack of a significant difference in mosquito control between the treatment with both fish, which was stocked with one half of the amount of mosquitofish in the treatment with only mosquitofish, and the mosquitofish alone treatment indicates that the lower stocking rate may provide adequate control of larval mosquito populations. A previous study showed that mosquito abundance did not differ significantly when mosquitofish were stocked in the spring at rates of 4 kg/ha and 1 kg/ha (Walton and Mulla 1991). Another study of 2 stocking rates, 1.1 kg mosquitofish/ha and 3.4 kg mosquitofish/ha, concluded that the lower rate was effective for controlling mosquito larvae in rice fields (Kramer et al. 1988). Mosquitofish biomass increased approximately 1%/day in fish ponds (Coykendall 1977). In our studies, mosquitofish biomass after 6 wk during the summer was, on average, approximately one half of that for a similar period during the spring, 54 vs. 104 kg/ha. Within each study, final mosquitofish biomass in the 2 treatments was roughly equivalent. Even though stocking rates differed by almost 2-fold for the 2 studies, the instantaneous rate of increase of mosquitofish biomass (wet weight) in the mosquitofish alone and the combined fish treatments averaged 0.081 and 0.095 g/g/day, respectively, during the summer and 0.079 and 0.087 g/g/day, respectively, during the spring. Our results support previous findings that a lower stocking rate of mosquitofish of approximately 1.5 kg/ha may be sufficient to achieve adequate larval control in a habitat relatively devoid of vegetative cover.

The stickleback was not effective in controlling larval mosquito populations in the Riverside ponds, whereas the mosquitofish provided significant levels of control whether stocked alone or concurrently with sticklebacks. As compared to mosquitofish alone, mosquito control was not significantly enhanced when both fish were stocked together. We further found that neither species of fish affected nontarget fauna, but caution that these experimental ponds are unlike many vegetated habitats into which fish may be introduced as larvivorous agents. Because the mesocosms are not representative of all natural field conditions, further investigation of the stickleback as a biological control agent for larval mosquito populations is warranted.

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