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COMPARISON OF MOSQUITO CONTROL PROVIDED BY THE ARROYO CHUB (*GILA ORCUTTI*) AND THE MOSQUITOFISH (*GAMBUSIA AFFINIS*)

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ABSTRACT. Two 6-wk trials were conducted in 28-m^2 earthen ponds to compare the efficacy of the arroyo chub, *Gila orcutti*, to the mosquitofish, *Gambusia affinis*, as a biological control agent for mosquitoes and a possible replacement for the mosquitofish in sensitive watersheds of southern California. The mosquitofish population growth rate was 1.73 times greater than the arroyo chub population growth rate; however, greater reproduction by the mosquitofish did not result in significantly better reduction of mosquitoes than was provided by the comparatively small populations of arroyo chub. On average across a 6-wk study in the spring, both larvivorous fishes reduced the abundance of 3rd and 4th instars by 4- to 5-fold compared to that observed in the control ponds that lacked fish but contained few invertebrate predators. The abundance of nontarget microinvertebrates in ponds containing the mosquitofish was only 7% of that in ponds containing the arroyo chub during the summer, but did not differ significantly between the fish species treatments when zooplankton was comparatively more abundant during the spring. Even though the number of individuals produced by each fish species during 6 wk in the spring was greater than for fish stocked in the summer, species-specific population growth rates in the spring study (individuals/individual/d; mosquitofish, 0.077; arroyo chub, 0.044) were only slightly higher than in the summer (individuals/individual/d; mosquitofish, 0.068; arroyo chub, 0.039) indicating that differences in the number of fish stocked contributed primarily to the differences in final population size between spring and summer studies. The arroyo chub is native to the South Coastal drainages in California and should be considered as a viable alternative to the mosquitofish for integrated mosquito management programs in riverine wetlands and sensitive watersheds of southern California.

KEY WORDS Larvivorous fish, biological control, *Culex*, native fish, riverine wetlands

INTRODUCTION

The mosquitofish, *Gambusia affinis* (Baird and Girard), can be a useful biological control agent for mosquitoes in particular habitats such as isolated pools, agricultural drainages, and small water bodies in urban environments; however, the mosquitofish may be less desirable for mosquito control than are native fish and insect predators in some natural settings (Moyle 2002). Environmental factors that affect mosquitofish abundance and effectiveness in controlling mosquitoes include the amount of vegetation, abundance of other prey, predators of the mosquitofish, water quality, and factors that regulate mosquitofish breeding cycles (Sawara 1974, Gratz et al. 1996, Swanson et al. 1996). Whereas the mosquitofish can be effective for controlling mosquitoes in nonvegetated or sparsely vegetated man-made impoundments, it is not effective as a mosquito larvivore in all aquatic habitats (Rupp 1996), and the addition of mosquitofish to natural habitats outside its native geographic range is regulated by law or discouraged because the mosquitofish is known to prey upon and competitively eliminate native fishes (Arthington and Lloyd 1989, Courtenay and Meffe 1989, Rupp 1996, Minckley 1999, Sheller et al. 2006). The mosquitofish also consumed the eggs and larvae of stream-dwelling amphibians in experiments carried out in structurally simple laboratory settings where alternative prey were rare (Gamradt and Kats 1996, Goodell and Kats 1999) and may adversely affect amphibian populations in some natural settings (Grubb 1972, Hayes and Jennings 1986; but see Lawler et al. 1999). A substitute for the mosquitofish is needed for settings where local biodiversity may be imperiled by the addition of a nonnative larvivorous fish (Offill and Walton 1999) and for mosquito control in natural settings near encroaching developments.

The arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann), is a good candidate as a biological control agent for mosquitoes in managed and natural wetlands because of its ecology and wide environmental preferences. The arroyo chub is native to the streams of southern California, specifically warm fluctuating streams (Wells and Diana 1975), and has been successfully introduced into several streams outside of its original distribution (Swift et al. 1993). Arroyo chub prefers backwaters that are slow moving and warm (10–24°C), have muddy to sandy bottoms, and are >40 cm deep (Wells and Diana 1975, Bell 1978, Moyle 2002). However, the arroyo chub can be found in other habitats that are adequate for its needs, ranging from reservoirs (Swift et al. 1993) to fast-moving streams (velocities ≥80 cm/s) with coarse bottoms (Bell 1978). The arroyo

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chub is quite tolerant of hypoxia, wide temperature fluctuation (Castleberry and Cech 1986), and fluctuating water conditions (Swift et al. 1993). It is a fractional spawner, breeding somewhat continuously from February through August, but mostly during June and July, in quiet, slow-moving areas where the water temperature is around 14–24°C (Tres 1992).

The arroyo chub is omnivorous; it eats algae, insects, and small crustaceans (Moyle 2002). The arroyo chub prefers eating invertebrates when they are abundant in the spring, but will readily eat algae in the winter when invertebrates are not abundant (Greenfield and Greenfield 1972). The arroyo chub's feeding preferences are somewhat similar to those of the mosquitofish (Swanson et al. 1996), but the arroyo chub is not known to consume amphibian larvae or other fishes. Arroyo chub fry spend 3–4 months feeding on invertebrates in emergent vegetation (Tres 1992), a habitat where mosquito larvae typically occur. It is also known to feed on caddisfly larvae and mollusks in the benthos of cool streams (Richards and Soltz 1986).

Here, the efficacy of the arroyo chub as a mosquito control agent was compared to that of the mosquitofish in 2 studies in earthen ponds in southern California.

MATERIALS AND METHODS

Study site

Two studies were carried out in earthen ponds (4 m × 7 m) at the University of California, Riverside, (UCR) Aquatic Research Facility. A 6-wk study was carried out from June 13 to July 26, 2005. The second study was conducted from April 10 to May 19, 2006. Ponds within 2 rows of 7 ponds per row were used for the studies. Six ponds (3 replicate ponds for each fish species) were used in 2005, and 9 ponds (3 replicates per each fish species and a control without fish) were used in 2006. Offill and Walton (1999) provide a detailed description of the study site. The ponds were devoid of emergent vegetation; 3 cinder blocks were added to the center of each pond to provide refuge for the fishes. Each pond was enriched with 0.65 kg of rabbit pellets (Nutriphase® Rabbit Formula; Pacific Coast Distributing, Inc., Phoenix, AZ) to promote colonization by insects 7 days before stocking fish. Water was supplied through a single pipeline from a reservoir, and water depth was maintained at 0.36 m by float valves.

Physicochemical factors

Water temperatures were measured using maximum-minimum recording thermometers (Markson Scientific, Inc., Del Mar, CA). Water temperatures were recorded every 48–72 h during the duration of each study in 1 northern pond (pond D6) and in 1 southern pond (pond C1). Thermometers were positioned vertically against one of the boards that defined the perimeter of each pond.

Water quality measurements were made 3 wk after the start of each experiment. Specific conductance was measured using an electronic sensor (TDSTester1™; Oakton Instruments, Vernon Hills, IL). Nitrate (in 2005 only) and ammonia concentrations were measured using ion-specific electrodes (nos. 9307 and 9512, respectively; Orion Research, Inc., Beverly, MA). Biochemical Oxygen Demand (BOD_5) in ponds in the 2006 study was measured following American Public Health Association (1995) protocol; an ion-specific electrode (no. 9708; ThermoOrion, Inc., Waltham, MA) was used to measure changes in the dissolved oxygen concentration. Chlorine and pH were measured using a pool water test kit (Taylor Technologies, Inc., Sparks, MD).

Mosquitoes and nontarget invertebrates

Four 350-ml dip samples were taken twice weekly near the corners of each pond to monitor the abundance of mosquitoes and nontarget organisms. The dip samples from each pond were combined using a concentrator cup (mesh opening = 153 μm). Dip samples were taken between 1300 and 1400 h. Specimens were preserved in alcohol (final concentration was approximately 50%). In the laboratory, immature mosquitoes were categorized into 3 subpopulations: 1st and 2nd instars, 3rd and 4th instars, and pupae. Late instars were identified to species using Meyer and Durso (1999).

Nontarget invertebrates were separated into microinvertebrate (zooplankton) and macroinvertebrates (nonculicine aquatic insects). Microinvertebrates were separated into cladocerans, copepods, and ostracods. Macroinvertebrates were keyed to at least the family level using the keys of Merritt and Cummins (1996).

Fish production

Arroyo chubs were collected from a captive population maintained by the Riverside-Corona Resource Conservation District (RCRCD) and transported to a holding pond at the UCR Aquatic Research Facility in spring 2005. The chubs and the offspring produced between the stocking of the holding pond and the beginning of the study were used in the 1st experiment. In 2005, mosquitofish were obtained from the Northwest Mosquito and Vector Control District (Corona, CA). Fish were added to the ponds on June 14.

In 2006, reproductively mature arroyo chub were collected from the RCRCD rearing facility and placed into treatment ponds on April 10, 2006. Mosquitofish were collected from a constructed treatment wetland in San Jacinto, CA (Walton et al. 1998), using minnow traps lined with window screen and baited with dog food. Fish were transported under aeration in coolers, acclimated for >30 min in 50% water from field sites and 50% water from the UCR Aquatic Research Facility, and released into treatment ponds on April 10.

Treatments were assigned to ponds based on larval mosquito densities in pretreatment samples taken on June 13, 2005 or April 7, 2006. In 2005, 2 treatments (mosquitofish or arroyo chub) were distributed among 6 ponds so that the variation in mosquito abundance across treatments was equivalent. Arroyo chubs were stocked into each pond at a rate of 4.5 kg/ha (mean = 12.5 g/pond) as 4 reproductive and 35 larval chubs. Mosquitofish were stocked at 3.4 kg/ha (mean = 9.4 g/pond) as 4 large (approximately 1.25 g/female) gravid reproductive females among 18–25 fish. Because summer 2005 was in the second year of the West Nile virus outbreak in southern California and it was unknown whether the number of human infections would decline as it had done in other regions of the United States, we did not run a treatment without fish. The weight at stocking differed between the two fish species because arroyo chubs are naturally larger than mosquitofish.

In 2006, 3 treatments (mosquitofish, arroyo chub, or control without fish) were replicated in 3 ponds per treatment. Treatments were again distributed among the ponds so that the variation in mosquito abundance across treatments was equivalent. Thirty-one adult arroyo chub were stocked into each of 3 ponds at a rate of 13.2 kg/ha (mean = 37 g/pond). Thirty-one mosquitofish were stocked into each of 3 ponds at 3.6 kg/ha (mean = 10 g/pond). Four large (>1.25 g) gravid *Gambusia* were included in the reproductive individuals added to each pond.

Fish production was estimated at the end of each experiment by collecting fish from each pond using a seine (0.64-cm mesh openings). Three hauls were taken per pond. In 2005, all *G. orcutti* were weighed individually. A random sample of 100 mosquitofish was collected from each pond, and fish were weighed individually. The mosquitofish remaining in collections were weighed together for each pond. In 2006, 100 randomly selected *Gambusia* and *Gila* were weighed individually to construct weight class distributions for each fish species. The remaining adults and juveniles of each species were counted and weighed together by pond. Fish that were not captured with the seine were collected with a dip net after water levels declined and then weighed.

Statistical analysis

Statistical comparisons for the 2005 study were based on natural-log–transformed mean mosquito abundance per dip for each pond using a repeated measures analysis of variance (RM-ANOVA; SYSTAT Version 9.01®; SPSS, Inc., Chicago, IL). Because the variance for the abundance of mosquito pupae was not homogeneous among the treatments, a nonparametric Friedman test was used to test for differences between the 2 treatments in the 2005 experiment. A nonparametric RM-ANOVA on ranks was used to assess the significance of differences for the mosquito larval and pupal subpopulations among the 3 treatments of the 2006 experiment. Pairwise comparisons of mosquito larval abundance between the 3 different treatments was done using a Student-Newman-Keuls test in 2006.

Statistical comparisons for nontarget taxa were based on natural-log–transformed mean abundance of either microinvertebrates (crustacean zooplankton) or macroinvertebrates (nonculicine aquatic insects) using an RM-ANOVA.

The statistical significance of differences of fish production between the treatments containing fish were based on the mean biomass (wet weight) of each species and compared using a *t*-test. A Kolmogorov–Smirnoff test was used to compare weight class distributions of each fish species between the 2 experiments.

RESULTS

Physicochemical factors

Maximum water temperatures of the ponds on the south end of the 2 rows of ponds were approximately 3°C warmer than the northern ponds during summer 2005 (Fig. 1A). The northern ponds were partially shaded, whereas the southern ponds received full sun. The mean maximum and minimum water temperature of the warmer pond, C1, were 32.9°C and 22.6°C, respectively, during the study. The mean maximum temperature in the cooler pond, D6, was 29.6°C, but the mean minimum water temperature (22.8°C) was similar to that in pond C1. The maximum water temperature in pond C1 was 36.7°C during late July.

Unlike the 2005 study, the maximum water temperatures in spring 2006 were similar in the northern (pond D6) and southern (pond C1) ponds (Fig. 1B). The mean maximum water temperatures in the northern pond and southern pond were 28.2°C and 27.5°C, respectively, during spring 2006. The minimum water temperature during the 2006 study was similar in the 2 reference ponds (C1, 18.4°C; D6, 17.7°C). Maximum and minimum water temperatures in-

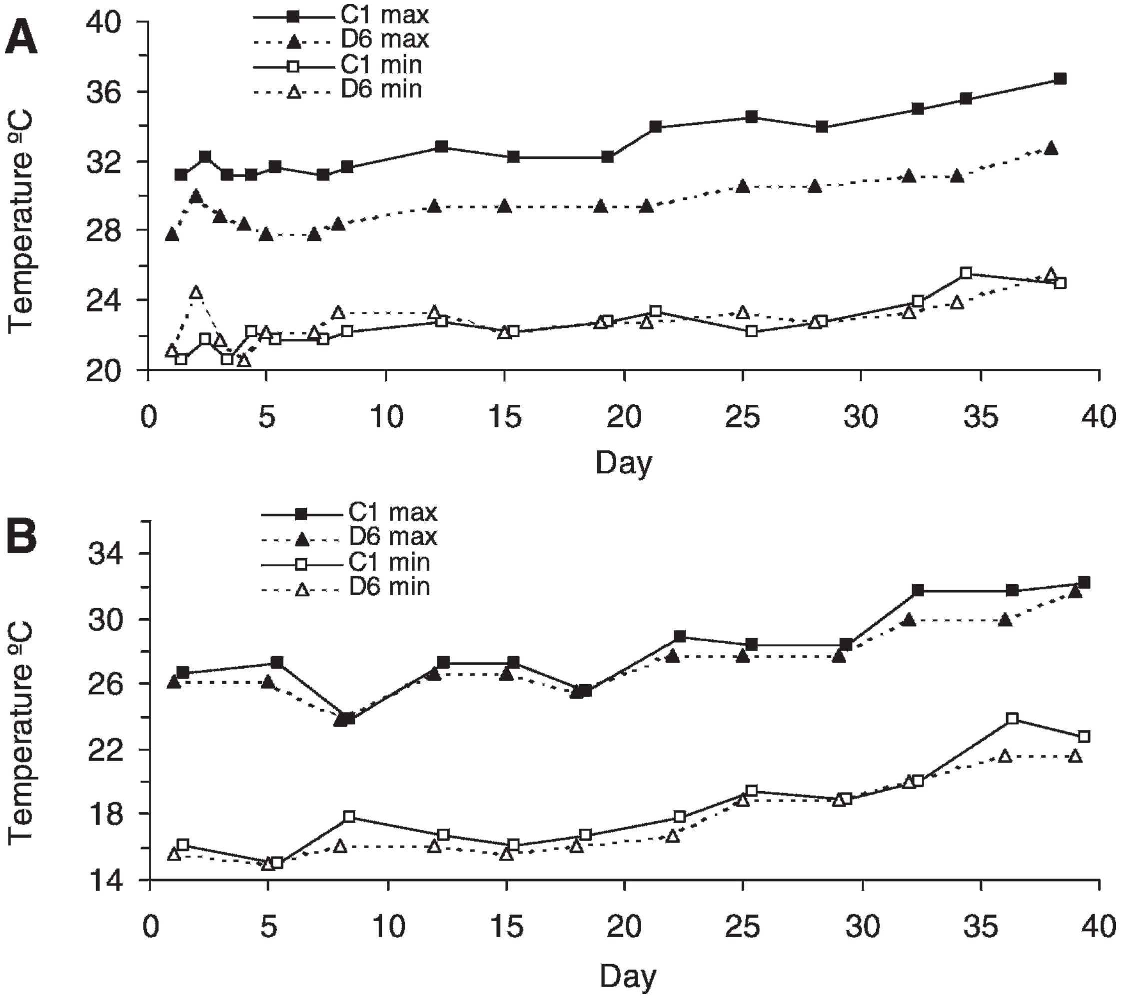

Fig. 1. Maximum and minimum temperatures in 2 ponds at the University of California Aquatic Research Facility, Riverside, CA, (A) from June 13 to July 26, 2005 and (B) from April 7 to May 19, 2006.

creased approximately 5.5°C during the 39-day experiment.

Other physicochemical factors did not differ markedly among ponds in the 2 fish treatments during the 2005 study. Mean (±SE) water conductivity across all of the ponds was 324.3 ± 11.6 μS/cm. The nitrate concentration in ponds with mosquitofish (65.1 ± 6.6 mg/liter) was similar to that in ponds with arroyo chub (60.6 ± 2.3 mg/liter). The chlorine concentration in the ponds was below the limit of detection (0.5 mg/liter). The pH in the ponds was approximately 7.8.

Mean water conductivity across all of the ponds during the 2006 study was 256.7 ± 3.7 μS/cm. The BOD$_5$ was similar for the 3 treatments (mean ± SE: control ponds 0.73 ± 0.08 mg/liter, arroyo chub ponds 0.76 ± 0.22 mg/liter, and mosquitofish ponds 0.66 ± 0.27 mg/liter). Chlorine concentration (<0.5 mg/liter) and pH (7.8) were consistent across the ponds. Ammonia concentration in the ponds was low (<0.001 mg/liter) and below the level stressful for fish (Swanson et al. 1996).

Mosquitoes and nontarget invertebrates

The predominant mosquito species collected during the 2005 study were *Culex stigmatosoma* Dyar (58% of 3rd and 4th instars) and *Culex tarsalis* Coq. (42% of late instars). Only 1 *Culex erythrothorax* Dyar larva and 1 *Anopheles* sp. larva were collected during the 2005 experiment.

Both fish species provided an equivalent level of control for larval (1st and 2nd instars, RM-ANOVA: F = 0.44, df = 1, 4, $P = 0.55$; 3rd and 4th instars, RM-ANOVA: F = 0.04, df = 1, 4, $P < 0.86$) and pupal (Friedman's test: $\chi^2 = 13.44$, df = 11, $P < 0.265$) mosquito subpopulations. The mean abundance of 1st and 2nd instars in both

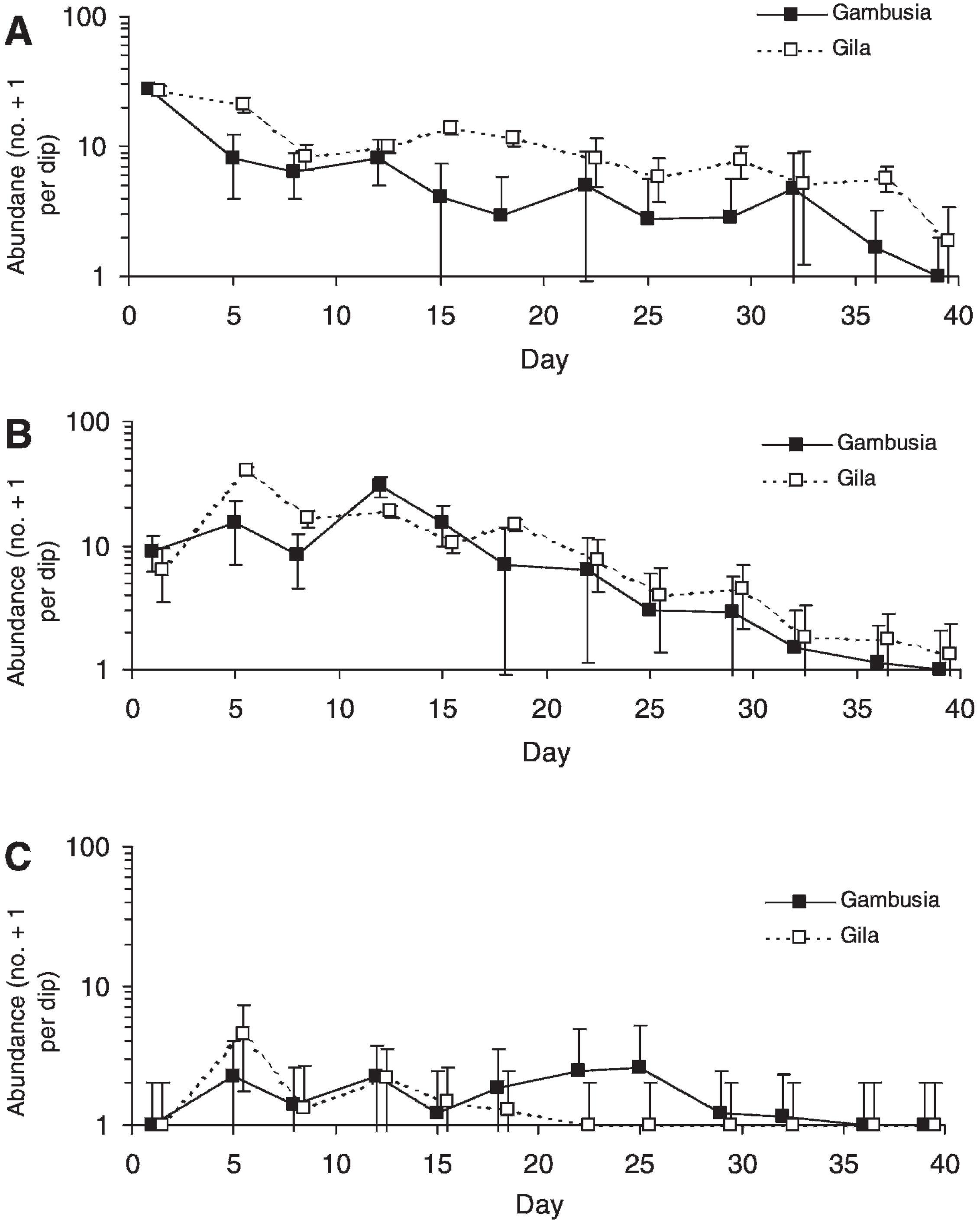

Fig. 2. Abundance (mean ± SE) of mosquito subpopulations in dip samples from ponds containing larvivorous fish during the period from June 13 to July 26, 2005: (A) 1st and 2nd instars, (B) 3rd and 4th instars, (C) pupae. Points are offset horizontally to facilitate illustration.

fish treatments fluctuated at low levels between day 7 and day 36, and then declined to ≤1 larva per dip by day 39 (Fig. 2A). The abundance of 3rd and 4th instars decreased slowly in both treatments after day 12, declining from approximately 20–30 mosquitoes per dip to ≤3 mosquitoes per dip after day 30 (Fig. 2B). Pupae were rarely collected during the experiment (Fig. 2C).

Table 1. Pairwise comparisons for the abundance of immature *Culex* spp. collected in dip samples in 3 treatments during 2006.

Stage[1]	Comparison	Difference of means or ranks	q[2]	$P < 0.05$
LI-LII	*Gila orcutti* vs. *Gambusia affinis*	4.00	1.63	No
	G. orcutti vs. Control	15.50	4.69	Yes
	G. affinis vs. Control	11.50	4.47	Yes
LIII-LIV	*G. orcutti* vs. *G. affinis*	3.50	1.43	No
	G. orcutti vs. Control	11.00	4.49	Yes
	G. affinis vs. Control	14.50	4.19	Yes

[1] L, larval instar.

[2] Student-Newman-Keuls statistic.

In 2006, the predominant mosquito species collected was *Cx. tarsalis* (mean relative abundance 99%). Only a few *Cx. stigmatosoma* larvae were collected.

Both fish species significantly reduced the abundance of larval mosquitoes (1st and 2nd instars: $\chi^2 = 11.021$, df = 2, $P < 0.004$; 3rd and 4th instars: $\chi^2 = 10.178$, df = 2, $P < 0.006$) compared to mosquito subpopulations in the control ponds without fish during spring 2006 (Table 1). First and second instars in the control ponds increased from ≤1 larva per dip on day 15, to nearly 15 larvae per dip on day 25 (Fig. 3A). The abundance of early instars in the ponds containing arroyo chub and mosquitofish was similar to that in the control ponds through day 15 but then remained at levels lower than the control ponds until the end of the experiment (Fig. 3A).

The abundance of *Culex* spp. 3rd and 4th instars in the control ponds was similar to the

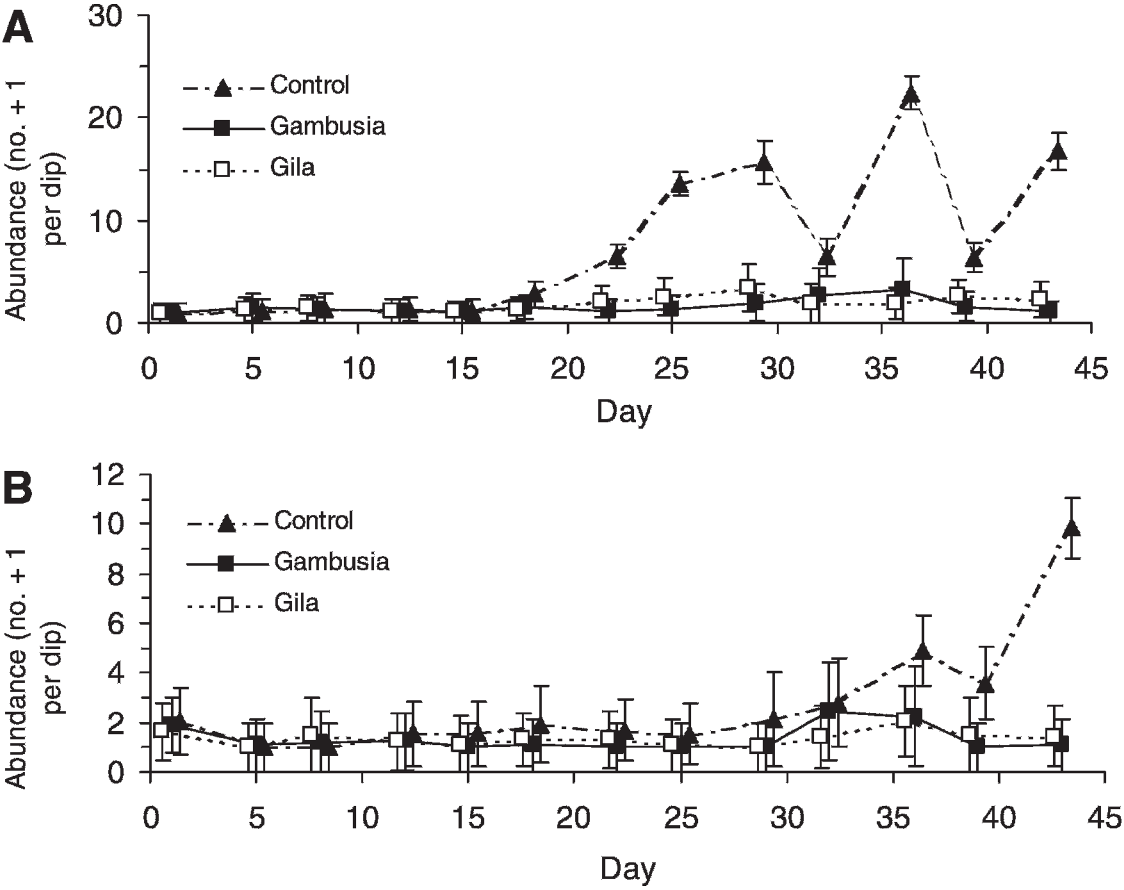

Fig. 3. Abundance (mean ± SE) of *Culex* spp. immature subpopulations from 3 treatments during the period from April 7, 2005 to May 19, 2006: (A) 1st and 2nd instars and (B) 3rd and 4th instars. Points are offset horizontally to facilitate illustration.

Table 2. Mean abundance (± SE) of nontarget invertebrates collected in dip samples among ponds containing and lacking larvivorous fish treatments during 2 experiments.

Nontarget group	Treatment *Gambusia affinis*	 *Gila orcutti*	 Control (no fish)	P[1]
2005 experiment				
Microinvertebrates	3.87 ± 1.75	53.99 ± 14.62		0.029
Macroinvertebrates	9.10 ± 4.07	9.06 ± 3.31		0.886
2006 experiment				
Microinvertebrates	244.43 ± 95.17	281.80 ± 31.97	201.53 ± 24.55	0.643
Macroinvertebrates	8.20 ± 1.51	9.50 ± 1.86	10.28 ± 1.59	0.515

[1] *P*-values from repeated measures analysis of variance.

ponds containing *G. orcutti* and *G. affinis* for about 1 month and then was higher than in the ponds containing fish. After day 32, late instars in the control ponds increased to 9 larvae per dip by the end of the experiment (Fig. 3B). The numbers of *Culex* 3rd and 4th instar collected from mosquitofish ponds and arroyo chub ponds remained at comparatively low levels (≤1 larva per dip) through day 43.

The abundance of mosquito pupae did not differ significantly among the 3 treatments during the 2006 study ($\chi^2 = 0.087$, df = 2, $P < 0.957$). After day 5, the abundance of *Culex* pupae was low throughout the experiment, except after day 35 in the ponds without fish, where the abundance of pupae increased as the comparatively large populations of larvae completed development.

The numbers of nontarget microinvertebrates in dipper samples differed significantly between the ponds with mosquitofish or arroyo chub during the 2005 experiment (Table 2). The abundance of microinvertebrates in the ponds stocked with the former species after day 8 was significantly lower than in ponds stocked with the latter (Fig. 4A). Microinvertebrates in ponds containing arroyo chubs were approximately 14 times more abundant than in the ponds containing mosquitofish (Table 2). The groups of macroinvertebrates collected were mostly rare (Table 3) and the abundance of nontarget insects did not differ significantly between the 2 fish treatments (Table 2).

In 2006, there were no significant differences in the numbers of nontarget microinvertebrates and macroinvertebrates in dipper samples among the 3 treatments (RM-ANOVAs, Table 2). Microinvertebrate abundance during 2006 (Fig. 4B) was greater than during 2005 and, unlike the 2005 study, did not differ significantly between ponds stocked with mosquitofish or arroyo chub. The most common nontarget invertebrates were again the microinvertebrates (Table 3). The other invertebrate groups were comparatively rare in 2006.

Fish production

There was no statistical difference in the weight class distributions for each fish species between the 2005 and 2006 studies (Kolmogorov–Smirnoff pairwise comparison, $P > 0.05$). The majority of individuals of both fish species weighed ≤1 g at the end of both studies (data from both studies combined: Figs. 5A, 5B).

Production of the 2 fish species differed significantly in 2005 (*t*-test, $P < 0.048$). The mean wet weight of individual mosquitofish was 0.43 ± 0.03 g at the end of the 2005 study. On average, each arroyo chub (0.64 ± 0.05 g) was 50% heavier than was an individual mosquitofish. At the end of the 2005 experiment, many more mosquitofish were produced per pond, and biomass after 6 wk (57.8 kg/ha; 161.73 ± 34.30 g/pond) was 12.4 times higher than for *Gila* (4.6 kg/ha; 13.00 ± 2.90 g/pond; Fig. 6A). Mosquitofish biomass increased 3.90 g/day and *G.* orcutti biomass increased only 0.10 g/day.

In 2006, a similar number of fish, rather than a roughly equivalent biomass, was added to ponds, and fish biomass after 6 wk did not differ significantly between the 2 fish treatments (*t*-test, $P < 0.990$). Most of the fish collected at the end of the experiment were small (mean ± SD: mosquitofish, 0.12 ± 0.02 g; arroyo chub, 0.46 ± 0.12 g) but, on average, each arroyo chub was nearly 3 times heavier than each mosquitofish. Fish biomass at the end of the experiment was >30 kg/ha per pond (mosquitofish: 33.14 kg/ha, 92.78 ± 15.66 g/pond; arroyo chub: 32.99 kg/ha, 92.37 ± 15.80 g/pond; Fig. 6B). During spring 2006, biomass (wet mass) increased 2.12 g/day for mosquitofish and 1.41 g/day for arroyo chub.

Mosquitofish produced 19 times and 4 times more offspring than did arroyo chub in the summer and spring, respectively. Based on calculations using the total fish biomass per pond and the mean mass per individual for each study, the mean number of mosquitofish per pond was estimated to be 376 individuals (range based on the SE: 296–456) and 773 individuals (range: 643–904) at the end of the summer and spring studies, respectively.

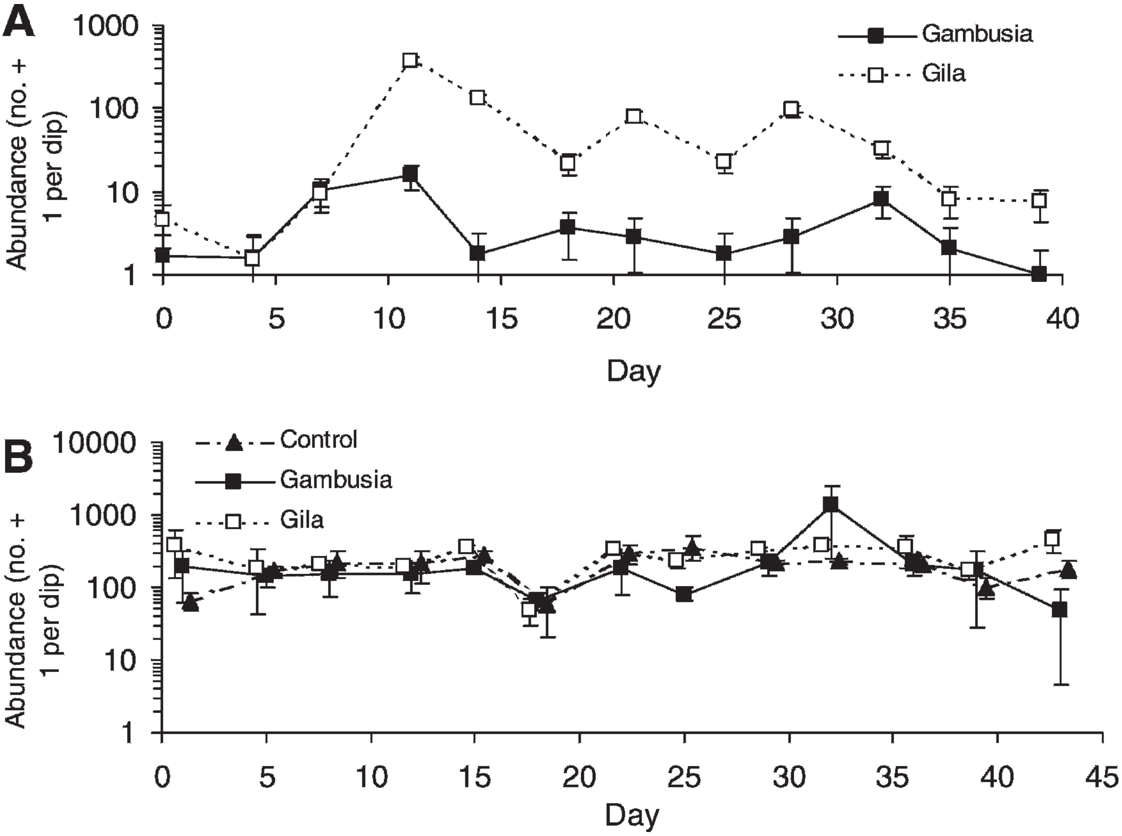

Fig. 4. Microinvertebrate abundance per dip sample (mean ± SE) taken (A) from June 13 to July 26, 2005, and (B) from April 7, 2005 to May 19, 2006. Points are offset horizontally to facilitate illustration.

Table 3. Nontarget taxa collected from experimental ponds at the University of California Aquatic Research Facility in Riverside, CA, from June 13 through July 22, 2005, and from April 10 through May 19, 2006.

	Abundance[1]	
Nontarget group	2005	2006
Anisoptera: Aeshnidae	R	R
Anisoptera: Libellulidae	R	R
Ceratopogonidae	R	R
Chironomidae	C	C
Cladocera	C	A
Copepoda	C	A
Corixidae	R	R
Dytiscid larvae	R	U
Ephemeroptera	R	R
Ephydrid larvae	R	R
Hydrophilid larvae	R	R
Laccophilus spp.	R	R
Notonectidae	R	R
Ostracoda	R	R
Veliidae	R	R
Zygoptera: Coenagrionidae	R	R

[1] A, abundant ($\geq$10,000 individuals collected); C, common (1,000 $\leq$ C $<$ 10,000 individuals collected); U, uncommon (100 $\leq$ U $<$ 1,000 individuals collected); R, rare ($<$ 100 individuals collected).

The mean number of arroyo chub per pond was 20 individuals (range: 15–25) and was estimated to be 200 individuals (range: 166–235) at the end of the summer and spring studies, respectively.

The species-specific population growth rate was similar in both studies, albeit populations of both fish species were growing slightly faster in the spring vs. the summer. If it is assumed that the fish populations were growing exponentially in the ponds during the 6 wk after stocking, then the intrinsic rate of increase of *G. affinis* was 1.73 times that for *G. orcutti*. The rate of population increase for the mosquitofish was 0.068 (range based on SD: 0.056–0.080) individuals · individual^{-1} · day^{-1} during summer 2005 and 0.076 (0.069–0.083) individuals · individual^{-1} · day^{-1} during spring 2006. The rate of population increase for the arroyo chub was 0.039 (0.038–0.040) individuals · individual^{-1} · day^{-1} during the summer study and 0.044 (0.037–0.051) individuals · individual^{-1} · day^{-1} during the spring study.

DISCUSSION

The mosquitofish and the arroyo chub provided equivalent levels of mosquito control in

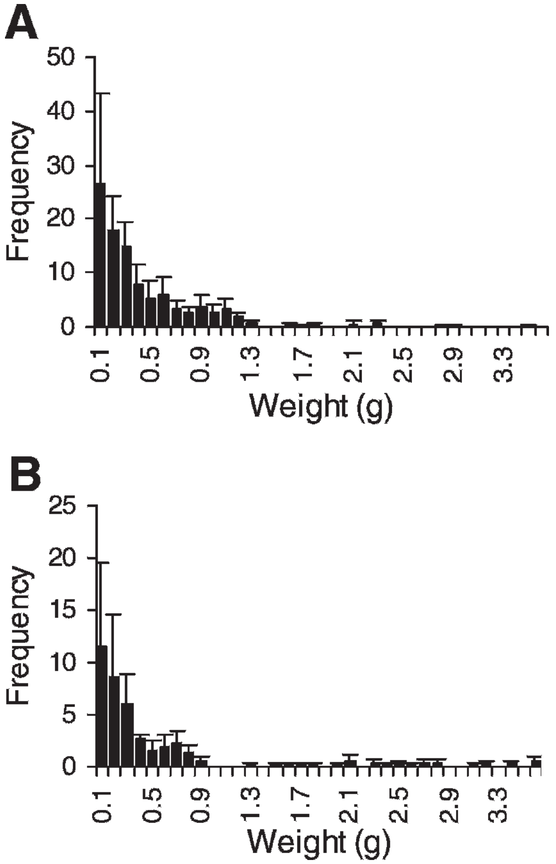

Fig. 5. Relative abundance of weight (wet weight) classes of 2 larvivorous fishes (A, *Gambusia affinis*; B, *Gila orcutti*) from the 2005 and 2006 experiments at the University of California Aquatic Research Facility, Riverside, CA. The mean ± SD is illustrated for each weight category.

earthen ponds during two 6-wk studies. The mosquitofish was more prolific than the arroyo chub; however, greater reproduction by mosquitofish did not result in significantly better reduction of mosquitoes than was provided by the comparatively smaller populations of arroyo chub. Both larvivorous fishes provided better mosquito control than did the small numbers of invertebrate predators in the control ponds that lacked fish. High water temperatures (>33°C) during a previous study at this site significantly reduced the survival of the threespine stickleback (*Gasterosteus aculeatus* L.), an alternative larvivorous fish to mosquitofish (Offill and Walton 1999), but both fish species used in the current study survived high water temperatures without significant mortality.

Hot weather, which decreased mosquito ovipositional activity at the site (Van Dam 2007), and density-dependent predation pressure on immature mosquitoes from the larvivorous fish populations were probably important factors contributing to the decline in mosquito populations observed in July 2005. A steep drop in the abundance of early instars in the ponds stocked with mosquitofish occurred during the last 3 sampling dates of the 2005 study. After day 10 of the 2005 experiment, microinvertebrates in ponds stocked with mosquitofish were 14-fold less abundant than in the ponds with arroyo chub, suggesting that the former species was consuming microinvertebrates preferentially to the immature mosquitoes. The larger number of young mosquitofish compared to arroyo chub may explain the more rapid decline in the early-stage larval mosquito populations in ponds stocked with mosquitofish. Immature-mosquito abundance also decreased in the ponds stocked with arroyo chub, although the decline in numbers was not as rapid as in the mosquitofish ponds. When alternative prey, such as Cladocera, is abundant, small mosquitofish preferentially feed upon zooplankton (Bence 1988). Furthermore, large mosquitofish prefer feeding on predatory insects compared to mosquito larvae (Bence and Murdoch 1986, Bence 1988). Therefore, when a mosquitofish population is at an intermediate level of buildup after stocking and alternative prey are abundant, larvivorous mosquitofish may not reduce immature mosquito abundance significantly (Bence 1988). In 2005, it is likely that the natural attenuation of the mosquito population after initial flooding, the initially small larvivorous fish population and abundant alternative prey, and an initially prolific mosquito population resulted in a low level of mosquito control and the gradual decline in the mosquito population seen in both treatments

In contrast to the 2005 study, mosquito populations did not decline continuously across the 2006 experiment in the treatments with larvivorous fishes. The resurgence of mosquito populations in control ponds after 2 wk was likely attributable to the vernal peak period of *Culex* oviposition. The abundance of *Culex* early instars in the fishless control ponds increased appreciably after day 15, indicating an intensification of mosquito ovipositional activity that, relative to the initial 2 wk of the study, remained high for the next 4 wk of the study. Water quality variables (ammonia, BOD_5, chlorine, nitrate, pH, specific conductance) in the ponds were equivalent across the 3 treatments in 2006 and did not contribute to differences of mosquito abundance between the treatments containing or lacking larvivorous fish. Van Dam (2007) found that egg laying by *Cx. tarsalis* on water conditioned by mosquitofish was lower than on aged tap water alone in the laboratory. This might partially explain the differences of mosquito abundance observed in the ponds containing fish vs. lacking fish in 2006.

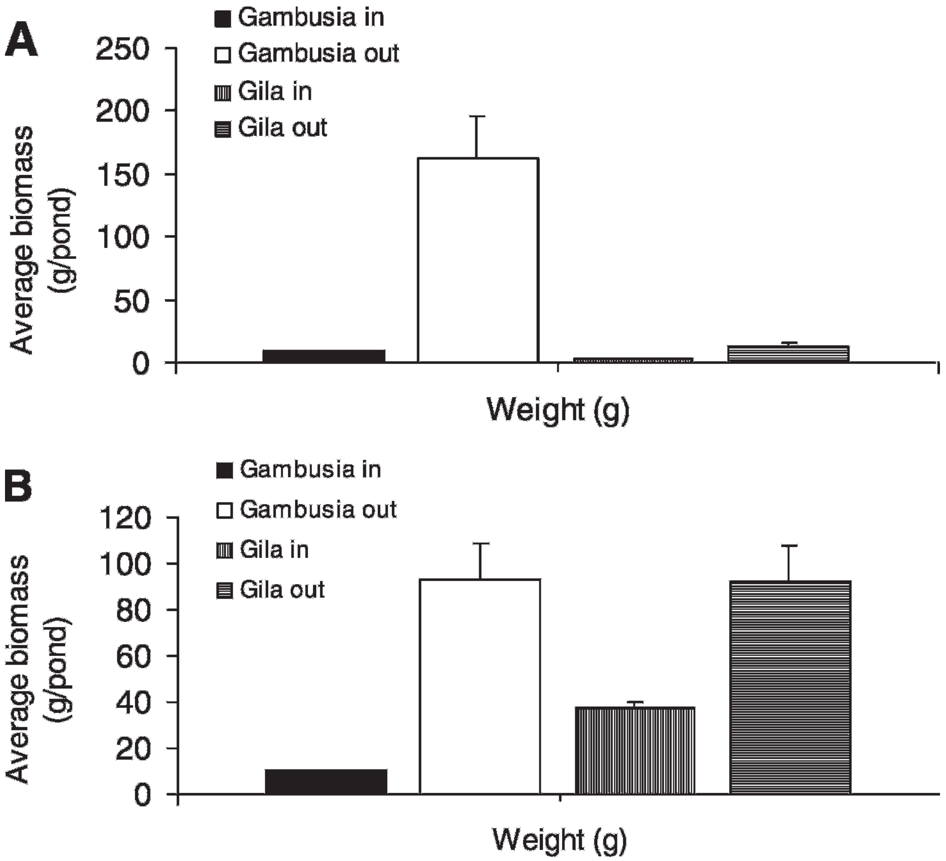

Fig. 6. Fish biomass at stocking and after 6 wk for 2 studies at the University of California Aquatic Research Facility, Riverside, CA, (A) 2005 experiment and (B) 2006 experiment.

Microinvertebrate abundance did not differ among the 3 treatments during spring 2006, unlike the 2005 study, in which microinvertebrate abundance in ponds stocked with *Gambusia* was on average 7% of that in the ponds stocked with arroyo chub. Yet, there was a steep decline in the number of microinvertebrates in ponds containing mosquitofish during the last week of the 2006 experiment. Microinvertebrate abundance during the 2006 experiment was much higher compared to the 2005 study (cf. Figs. 4A, 4B) and may have played a role in dampening the effect of *Gambusia* predation on microinvertebrates until the end of the experiment when *Gambusia* were at their highest density per pond.

Biomass production for each fish species differed between treatments and years. Mosquitofish production during 6 wk in summer 2005 was nearly twice that recorded for a similar period in spring 2006 (final mean biomass: 57.8 g/ha vs. 33.1 g/ha). The lower mosquitofish production during spring 2006 was perhaps caused by the direct effect of cooler spring temperatures on mass-specific growth rates. While the mosquitofish population size in the 2006 study was greater than in 2005, the abundance of alternative prey (i.e., zooplankton) was nearly 60-fold that in the 2005 experiment; therefore, strong intraspecific competitive interactions were not likely to have caused the lower mass-specific production during spring 2006.

Arroyo chub production showed a trend opposite to that of mosquitofish, with greater production during the spring than in the summer. The addition of a greater number of adult fish in spring 2006 than in summer 2005 (31 vs. 4) contributed to the differences in production between years. In 2006, the larger number of adult arroyo chub reproduced and biomass in ponds increased more than 7-fold (33 kg/ha vs. 4.6 kg/ha) compared to 2005.

Even though the number of individuals produced by each fish species differed between the 2 studies, the species-specific population growth rate was similar in both studies, indicating that differences in the stocked populations contributed primarily to the differences of population size between spring and summer studies. The ability of arroyo chub to increase to sufficient numbers to control mosquitoes is linked to the time of the year of stocking, the reproductive status of the stocked fish, and the number of fish stocked. This study indicated that stocking the arroyo chub in the spring resulted in greater production than stocking during the summer, and given the 3-fold difference in wet mass between the adults of the two fish species, the mass-specific stocking rate for arroyo chub is higher than for the mosquitofish. Various studies have suggested different spring stocking levels for mosquitofish ranging between 1 and 4 kg/ha (Kramer et al. 1988, Walton and Mulla 1991, Offill and Walton 1999). For mosquitofish, it is critical to stock fish in the early spring because winter die-off can significantly reduce mosquitofish populations (Walton 2007). The arroyo chub should be stocked in early spring (March or early April) at the beginning of their annual reproductive period (Tres 1992), and an area-specific mass >10 kg/ha may be required if mosquito control is needed soon after stocking fish; further studies are needed.

Our studies suggest that native arroyo chub may be a viable replacement for nonnative mosquitofish in natural aquatic ecosystems in the South Coastal drainage system of southern California, especially in riverine wetlands used to improve water quality and provide habitat for endangered species. The arroyo chub provided levels of mosquito control similar to that provided by the mosquitofish and did not significantly reduce nontarget invertebrate populations during both 6-wk studies. Unlike the stickleback, which also has been considered as an alternative larvivorous fish to the mosquitofish, the arroyo chub is capable of withstanding high summer water temperatures. The arroyo chub is likely to provide meaningful levels of mosquito control in wetlands associated with rivers and streams in southern California because the arroyo chub is better adapted to life in slow and fast moving water than is the mosquitofish. Movement of fish among watersheds is often restricted because of genetic differences among populations, potential transfer of pathogens and parasites, and the presence of other sensitive species; therefore, coordination of vector control activities using the arroyo chub will require coordination with federal and state agencies responsible for the management of natural resources. Further studies are needed to assess the sustainability of chub populations and the efficacy of the arroyo chub for mosquito control in wetlands.

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