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COMPARISON OF GILA TOPMINNOW AND WESTERN MOSQUITOFISH AS BIOLOGICAL CONTROL AGENTS OF MOSQUITOES

Michael R. Childs¹

ABSTRACT.—I compared acute tolerance of Gila topminnow, *Poeciliopsis occidentalis*, and western mosquitofish, *Gambusia affinis*, to ammonia and high water temperature and also compared diet, food selectivity, and impact on invertebrate populations to evaluate the potential for each species to effectively control mosquitoes (Culicidae) in wetland habitats. Critical thermal maxima differed among species, sexes, and lifestages, but these differences were likely not biologically significant due to their small magnitude and the ability of wild populations of both species to adapt to high temperatures. In 24-hour tests, *G. affinis* displayed about twice the ammonia tolerance as did *P. occidentalis*. However, long-term survival in habitats with high ammonia concentrations is questionable, thus reducing the perceived benefit of using *G. affinis* for mosquito abatement in these habitats. Diet overlap was significant between sexes within species but was not significant between species. However, both species exhibited significant dietary selection for mosquito larvae and pupae under experimental conditions. Shared dietary preference for mosquito larvae and pupae, in addition to similarity in temperature tolerance between species, suggests that *P. occidentalis*, a native and historically widespread species, can be used in place of nonnative *G. affinis* for mosquito control. There is no evidence to suggest that nonnative *G. affinis* is better suited for mosquito abatement, and since *G. affinis* is replacing *P. occidentalis* throughout the native species' former range, it should no longer be introduced into the Gila River basin for mosquito control. However, managers who would use *P. occidentalis* for mosquito abatement must take care not to interfere with conservation efforts by disrupting the genetic structure of natural populations of *P. occidentalis*.

Key words: *Poeciliopsis occidentalis*, *Gambusia affinis*, mosquito control, diet selection, ammonia tolerance, temperature tolerance.

The American Southwest is an arid region with a rapidly growing human population. As a result of rapid growth, wastewater treatment facilities and associated artificial wetlands have proliferated. Some wetlands are noted for harsh environmental extremes, particularly high unionized ammonia levels (King et al. 1999, Boyle and Fraleigh 2003) and high water temperatures. Wetlands are also breeding grounds for mosquitoes (Culicidae) which can transmit diseases such as West Nile virus, encephalitis, and malaria. Negative environmental effects of pesticides and increasing resistance of mosquito larvae to pesticides have renewed interest in use of biological control agents (Haas and Pal 1984). Use of larvivorous fishes is the oldest and most popular biological method for reducing mosquito larvae populations (Homski et al. 1994 and references therein).

The western mosquitofish, *Gambusia affinis*, has been introduced worldwide for biological control of mosquitoes. The species is tolerant of low oxygen levels ($0.5 \text{ mg} \cdot \text{L}^{-1}$; Homski et al.

1994) and capable of developing resistance to temperature extremes (Otto 1973) and chemical toxins (Angus 1983). *Gambusia affinis* can be effective at reducing mosquito populations (Gall et al. 1980, Castleberry and Cech 1990). However, because *G. affinis* is predominantly found in open water (reviewed by Meffe and Snelson 1989), many native fishes are just as effective or more effective at mosquito control in natural and artificial habitats with dense vegetation (Danielson 1968, Walters and Legner 1980, Homski et al. 1994).

Due to negative impacts on indigenous fishes, the World Health Organization has recommended caution when introducing *G. affinis* outside its native range (WHO 1982). In Arizona, nonnative *G. affinis* competes with and preys upon Gila topminnow, *Poeciliopsis occidentalis*, and is replacing this species in its native range (reviewed by Minckley et al. 1977, Meffe et al. 1983, Minckley et al. 1991). Prior to 1940, *P. occidentalis* was one of the most common fishes in the lower Colorado River

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basin (Hubbs and Miller 1941), but by 1967 it was rare and listed as endangered by the U.S. Fish and Wildlife Service (Minckley et al. 1991). At present, *P. occidentalis* is confined to 14 natural populations in southern Arizona (Weedman 1999) and reestablished populations in natural and manmade habitats in the wild. Use of *P. occidentalis* for mosquito control in artificial wetlands in Arizona would provide more refuge populations and would also eliminate or greatly reduce the need to stock nonnative *G. affinis* (Clarkson and Childs 2001).

I compared several environmental attributes of *P. occidentalis* and *G. affinis*, including acute tolerance to un-ionized ammonia and high water temperature, diet, food selectivity, and impact on invertebrate populations, to assess the mosquito-control potential of each species in wastewater treatment wetlands.

METHODS

Fish were collected for temperature and ammonia tolerance experiments during summer and autumn of 1999 using standard minnow traps. I collected *G. affinis* from earthen ponds at Bubbling Ponds Hatchery near Cornville, Arizona, and collected *P. occidentalis* from an earthen pond (Ayer Lake) at Boyce Thompson Arboretum near Superior, Arizona. Fish of both species were treated for Asian tapeworm, *Bothriocephalus acheilognathi*, with Praziquantel (Sigma Chemical Corp., Saint Louis, Missouri) at 0.67 ppm for 24 hours. I held *G. affinis* and *P. occidentalis* at Bubbling Ponds Hatchery in separate outdoor 1900-L fiberglass tanks that were continuously aerated and received flow-through water from an artesian spring (110 ppm CaCO₃, 18.5°C, pH 7.6). I fed fish once or twice each day ad libitum with Aquatox[®], a commercial flake food (Aquatic Ecosystems Inc., Apopka, Florida). All fish were held for at least 1 week and were not fed for 24 hours prior to use in laboratory experiments, which were conducted at Bubbling Ponds Hatchery.

Critical Thermal Maximum

I conducted critical thermal maximum experiments between 28 June and 15 September 1999. I investigated effects of age and sex on critical thermal maximum, because these factors have been shown to affect temperature tolerance in *G. affinis* (Krumholz 1948, Hagen 1964, Johnson 1976). I randomly selected 10 fish for

each test (adult males, adult females, and unsexed juveniles of each species tested separately) and transferred them indoors to an aerated 500-mL test beaker containing water at 20°C. The beaker was then placed in a 33-L, deep-chamber water bath (VWR Scientific, Inc., San Francisco, California), and water temperature was increased at a rate of approximately 0.1°C · min⁻¹. I recorded the temperature at which each fish lost equilibrium and used the mean temperature recorded for all 10 fish in a test as an independent measure of critical thermal maximum. I repeated experiments 5 times for each test group and compared critical thermal maxima among groups (each "group" was represented by a single species and sex or life-stage) using 1-way analysis of variance (ANOVA) and Duncan's multiple range post hoc comparisons (SPSS 2002).

Un-ionized Ammonia Toxicity

I determined median lethal concentrations of un-ionized ammonia for both species using standard bioassay procedures (24-hour tests at constant water hardness, pH, and temperature) between 19 October and 5 December 1999. I adjusted un-ionized ammonia concentration by addition of ammonium chloride to water buffered with sodium phosphate (Ashe et al. 1996) and maintained water at ambient room temperature (20°C). I adjusted pH to 7.00 using hydrochloric acid or sodium hydroxide solutions as needed and measured total ammonia concentration (ppm) with an Orion Model 370 meter (Orion Research, Inc., Beverly, Massachusetts). Un-ionized ammonia concentrations were calculated from tables in Emerson et al. (1975).

I conducted a preliminary study to determine the lethal range of 24-hour un-ionized ammonia tolerance separately for males and females of each species. Juveniles of each species were unavailable at the time of testing. I prepared 8 separate 38-L aquaria with un-ionized ammonia concentrations of 0, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 ppm and placed 5 fish in each tank. I assessed mortality after 24 hours and used the minimum concentration of un-ionized ammonia that killed all 5 test fish in a tank to determine the upper lethal range of 24-hour tolerance. *Gambusia affinis* males and females demonstrated a higher tolerance to un-ionized ammonia than *P. occidentalis*, so I

conducted a second preliminary test on *G. affinis* and adjusted concentrations to 0, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2 ppm.

I conducted un-ionized ammonia-tolerance tests in exactly the same manner as preliminary tests. Each test consisted of 7 experimental aquaria and 1 control (buffered, but no ammonia), with each tank containing 5 test fish. The 7 experimental aquaria contained different un-ionized ammonia concentrations that encompassed the lethal range of 24-hour un-ionized ammonia tolerance determined in the preliminary studies. I repeated each test 5 times, using fish from outdoor holding tanks and determined median lethal concentrations for each species-sex combination by probit analysis (Finney 1971). I evaluated differences across groups by calculating estimates of relative median potency (SPSS 2002).

Diet

I conducted a preliminary feeding study during July 2000 to determine the diel period of maximum gut fullness. This information was collected to determine the time of day that fish exhibited peak feeding activity, and thus the best time of day to collect fish and invertebrate samples during a subsequent foraging study (see below). I collected *G. affinis* from ponds at Bubbling Ponds Hatchery and *P. occidentalis* from Ayer Lake at Boyce Thompson Arboretum. I collected 10 males and 10 females at 6-hour intervals for 24 hours using standard minnow traps, euthanized fish using tricaine methanesulfate (MS-222), and preserved fish immediately in 10% formalin. I estimated percent gut-fullness visually following dissection and determined sampling period (hour) with highest gut-fullness for each species-sex combination using 3-way ANOVA and Tamhane's T2 (equal variance not assumed) post hoc multiple comparisons (SPSS 2002).

The species \times sex ($F = 7.22$, $df = 1$, $P = 0.008$) and species \times hour ($F = 3.36$, $df = 3$, $P = 0.020$) interaction terms were significant, so effects of sex and hour on gut-fullness were evaluated in separate 2-way ANOVAs for each species. *Gambusia affinis* gut-fullness was higher for females than males ($F = 5.41$, $df = 1$, $P = 0.023$), but no diel variation was observed ($F = 1.12$, $df = 3$, $P = 0.348$). *Poeciliopsis occidentalis* gut-fullness did not vary between sexes ($F = 1.89$, $df = 1$, $P = 0.173$), but did vary by hour ($F = 9.90$, $df = 3$, $P < 0.001$).

Post hoc comparisons indicated *P. occidentalis* gut-fullness was higher at 1200 hours than at either 0000 hours or 0600 hours ($P = 0.004$ and 0.045, respectively) and was also higher at 1800 hours than at 0000 hours or 1200 hours ($P = 0.001$ and 0.029, respectively). As a result, I collected fish and invertebrate samples starting at 1800 hours during the foraging study.

Fish used in the foraging study were collected on 11 September and 12 September 2000. I again collected *G. affinis* from ponds at Bubbling Ponds Hatchery. Because *P. occidentalis* was scarce at Boyce Thompson Arboretum in September 2000, I collected *P. occidentalis* at unnamed drainage #68 adjacent to Mesquite Draw, near Tortilla Flat, Arizona (Sec 1, T2N, R9E, USGS Mormon Flat Dam Quadrangle). This population of *P. occidentalis* is derived from the Boyce Thompson Arboretum stock. I held each species in 2 separate outdoor 1900-L fiberglass tanks at Bubbling Ponds Hatchery that were continuously aerated and received flow-through artesian spring water. I did not feed fish prior to introduction into experimental tanks (5–6 days).

I used 6 outdoor 1900-L fiberglass tanks located in a fenced and shaded enclosure at Bubbling Ponds Hatchery as artificial wetland habitats: 2 for *P. occidentalis*, 2 for *G. affinis*, and 2 for control. On 15 August 2000 I added equal volumes of sand substrate at a uniform depth (5 cm) to each tank and filled each with artesian spring water to a depth of approximately 30 cm. Each tank was fertilized with 125 g ammonium phosphate and 150 g alfalfa pellets to promote rapid algal growth and increase invertebrate densities. Tanks sat for 1 month before experimental treatments to allow natural colonization by mosquitoes and other flying insects and to allow invertebrate densities to stabilize. I sampled invertebrates (see below) from each outdoor tank at 1800–1900 hours on 16 September 2000, one day prior to initiating treatments (fish introduction), and intermittently thereafter (17, 20, 24, and 27 September, and 1 October 2000). I randomly assigned and introduced 100 adult fish of each species (50 males, 50 females) to separate tanks on 17 September 2000 at 0900 hours.

On each sampling date, 3 replicate samples of invertebrates were taken at random locations within each tank. I sampled the water column and upper benthic zone using 30-cm-diameter PVC pipe to form a column and swept the

encircled water column and upper benthic zone with a 250- μm -mesh dipnet for 30 seconds. I preserved invertebrate samples in 10% formalin and recorded counts and ash-free dry mass (AFDM) of each taxon. This data was converted to number and AFDM per m^2 benthic area sampled for each taxon. Beginning on 17 September 2000 I collected samples of 20 adult fish (10 males, 10 females) concurrently with invertebrate samples to assess diet and food selection. Collected fish were frozen immediately and replaced with equal numbers of adult fish to maintain fish density in experimental tanks. I analyzed the stomach proper (intestines anterior to the first anterior loop) and lower intestine separately for dietary analysis. I recorded counts and visually estimated gut-fullness and relative contribution of gut contents for stomach and lower intestine samples. Invertebrates were identified to subclass, order, or family. I did not count or weigh invertebrates <500 μm in size.

I used Schoener's (1968) index to assess diet overlap between sexes within species and diet overlap between species, using mean relative volume percentages of food categories that could be identified to taxon (body parts, detritus, and "other organic material" were excluded from analysis; see Results). Schoener's index ranges from 0 (no overlap) to 1 (complete overlap). Values greater than 0.6 and less than 0.4 are considered biologically significant, representing overlapping and differential use of resources, respectively (Schoener 1968, Ross 1986). I used Pearre's (1982) prey selection index to evaluate selectivity by *G. affinis* and *P. occidentalis* for diet items that could be identified and counted. The index ranges from -1 to 1, with a value of 0 representing no selection. The index is derived from the chi-square formulation and can therefore be tested for statistical significance (Pearre 1982).

RESULTS

Critical Thermal Maximum

Critical thermal maxima differed among species, sexes, and lifestages ($F = 27.66$, $df = 5$, $P < 0.000$). Female *G. affinis* exhibited a lower critical thermal maximum (36.7°C) than any other group, while male *G. affinis* and female *P. occidentalis* exhibited the highest critical thermal maxima at 38.6°C and 38.3°C, respectively (Table 1). As test temperatures approached

the lethal limit, behavioral differences also occurred between species. All adult *G. affinis* congregated near the water surface, whereas all adult *P. occidentalis* attempted to jump out of the test chamber, a behavior never exhibited by *G. affinis*.

Un-ionized Ammonia Toxicity

Gambusia affinis was more tolerant of un-ionized ammonia than *P. occidentalis*, and female *G. affinis* were more tolerant of un-ionized ammonia than were males (Table 2). Male and female *P. occidentalis* did not differ in tolerance to un-ionized ammonia.

Diet

I observed a high proportion of empty stomachs during the foraging study (54% for *G. affinis*, 40% for *P. occidentalis*) and therefore pooled stomach and lower intestinal diet data prior to analysis.

Schoener's index (averaged across sampling dates) indicated significant diet overlap between male and female *P. occidentalis* (0.62) and between male and female *G. affinis* (0.75). Therefore, I summarized species' diets without reference to sex (Table 3). Diet overlap between species (sexes lumped together) was not significant (Schoener's index = 0.46). Overall, I documented 33 food categories for *G. affinis* and 22 food categories for *P. occidentalis*. Some taxa were lumped together (e.g., "other insects") to condense Table 3. Data for these taxa are available from the author.

Mosquito larvae and pupae dominated diets of *P. occidentalis* and *G. affinis* on 17 September 2000, when fish were first introduced into experimental tanks, and frequency of occurrence of mosquito larvae in diets was identical (45%) for both species on that date. Diets of *P. occidentalis* and *G. affinis* shifted from mosquitoes (Culicidae) to midges (Chironomidae) as the experiment progressed (Table 3). Even more pronounced, however, was the temporal shift in *P. occidentalis* diet from invertebrates to "other organic material" (OOM), which was comprised mostly of filamentous algae and, to a lesser extent, digested material (based on compound microscopy).

Gambusia affinis exhibited cannibalism on juvenile fish during the foraging study (Table 3). Juvenile fish were available as diet items to both *G. affinis* and *P. occidentalis* as a result

TABLE 1. Mean critical thermal maxima ($^{\circ}\text{C}$) for *Gambusia affinis* males (GA-M), females (GA-F), and juveniles (GA-J), and *Poeciliopsis occidentalis* males (PO-M), females (PO-F), and juveniles (PO-J). Homogeneous subsets of means are shown in columns, and are based on Duncan's multiple range post hoc comparisons (SPSS 2002). Standard errors are shown. The procedure corrects for multiple tests (table-wide $\alpha = 0.05$). Significance of each comparison is shown in the bottom row.

Group	n	$\bar{x} \pm s_{\bar{x}}$				
		1	2	3	4	5
GA-F	5	36.7 \pm 0.1				
PO-M	5		37.3 \pm 0.2			
PO-J	5			37.8 \pm 0.2		
GA-J	5			38.0 \pm 0.2	38.0 \pm 0.2	
PO-F	5				38.3 \pm 0.1	38.3 \pm 0.1
GA-M	5					38.6 \pm 0.1
SIGNIFICANCE		1.000	1.000	0.380	0.097	0.113

TABLE 2. Un-ionized ammonia tolerance for adult *Gambusia affinis* and *Poeciliopsis occidentalis*. Median lethal concentrations after 24 hours were determined using probit analysis and are expressed in ppm un-ionized ammonia nitrogen. Significant differences ($\alpha = 0.05$) between median lethal concentrations are indicated with superscripted letters. Ninety-five percent confidence intervals are also shown.

	Tests	n	Mean length (mm)	Mean weight (g)	Median Lethal Concentration (ppm)	95% C.I.
<i>GAMBUSIA AFFINIS</i>						
Female	6	240	47.8	1.190	2.57 ^z	2.41–2.74
Male	5	200	31.1	0.267	2.10 ^y	1.96–2.24
<i>POECILIOPSIS OCCIDENTALIS</i>						
Female	5	200	46.0	1.033	1.04 ^x	0.95–1.14
Male	5	200	32.4	0.308	1.18 ^x	1.08–1.29

of live births during the study. I observed no cannibalism by *P. occidentalis*.

Mosquito larvae and pupae abundance declined rapidly following introduction of both fish species into test tanks (Fig. 1). By 20 September 2000 (day 5 of the experiment), both fish species had depleted virtually all immature lifestages of mosquitoes, and mosquito AFDM remained <1% of total AFDM in tanks containing *G. affinis* and *P. occidentalis* for the remainder of the experiment. Mosquito larvae and pupae were present in control tanks throughout the study (Fig. 1). Ash-free dry weights of all taxa in experimental tanks are available from the author.

Early in the experiment both fish species fed selectively on mosquito larvae and pupae over other invertebrates (Fig. 2) when these lifestages were relatively abundant. Selection for mosquito lifestages (when available) was not always statistically significant but was always positive for both fish species during the foraging study. In contrast, both species avoided

chironomid larvae early in the experiment, but selected this prey item during the middle and end of the experiment (Fig. 3).

DISCUSSION

Poeciliopsis occidentalis is desirable for use as a biological control agent of mosquitoes in the Gila River Basin, Arizona, because it is native and was historically widespread (Hubbs and Miller 1941). Use of *P. occidentalis* for mosquito abatement is warranted because my results indicate that it feeds selectively on mosquito larvae and pupae over other invertebrate taxa and that it was equal in effectiveness to *G. affinis* at removal of immature lifestages of mosquitoes from experimental tanks. Although results from this diet study cannot be extrapolated to all possible environmental conditions, the 2 fish species are ecologically similar and reproductively prolific (reviewed by Minckley 1999). Moreover, statistical differences in critical thermal maxima between *P. occidentalis* and *G. affinis* are likely not biologically significant

TABLE 3. Relative volume and frequency of occurrence of prey items consumed by *Gambusia affinis* and *Poeciliopsis occidentalis* in outdoor tanks at Bubbling Ponds Hatchery. Taxa are listed in the left column. Taxon OOM represents "other organic material." Letters following taxa indicate life stage of food item: A = adult, L = larva, P = pupa, and J = juvenile.

	<i>Gambusia affinis</i>												<i>Poeciliopsis occidentalis</i>																	
	Relative Volume (%)						Frequency of Occurrence (%)						Relative Volume (%)						Frequency of Occurrence (%)											
	Sep	Sep	Sep	Sep	Sep	Oct	Sep	Sep	Sep	Sep	Sep	Oct	Sep	Sep	Sep	Sep	Sep	Sep	Sep	Sep	Sep	Oct	Sep	Sep	Sep	Sep	Sep	Sep	Oct	
Sample Date	17	20	24	27	1	17	17	20	24	27	1	28	17	20	24	27	1	17	17	20	24	27	1	27	17	20	24	27	1	1
Sample Size	49	52	70	49	36	38	38	40	40	40	40	40	53	65	64	62	67	39	39	39	40	40	39	39	39	40	40	40	40	39
Body Parts	6	8	14	9	1	8	21	21	43	35	10	10	4	2	5	6	<1	5	13	40	50	5	5	5	13	40	50	5	5	5
Insects																														
Diptera																														
Chironomidae	A	1	5	17	17	3	3	3	20	20	28	28	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	L	9	16	13	14	20	24	51	63	60	48	48	24	51	45	13	3	6	2	2	2	4	8	20	15	23	45	8	13	49
	P			14	4	2			45	13	3	3	2		5		1	2		2		1	1	5	5	8	8	8	13	13
Culicidae	A	1	1	1	6	6	3	3	5	5	5	5	5	5	5	2	2	5	5	5	2	2	2	5	5	5	5	5	5	5
	L	34	4	1	<1	1	45	8	8	3	<1	<1	45	<1	3	3	<1	35	<1	<1	<1	<1	<1	45	3	3	5	5	3	3
	P	11	4				24	5	5	5	3	3	7	<1	7	<1	<1	7	<1	<1	<1	<1	<1	18	3	3	5	5	5	5
Other	A	1	2	3	3	14	3	8	11	15	19	19	3	8	11	15	19	3	8	11	15	19	19	3	8	11	15	19	19	19
	L	1	1	1	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	P	1	2	<1			3	<1	3	3	3	3	3	<1	3	3	3	3	<1	3	3	3	3	3	<1	3	3	3	3	3
Ephemeroptera	A	1	1	1	2	3	3	3	3	3	10	10	3	3	3	3	10	3	3	3	3	3	3	3	3	3	3	3	3	3
	L	11	4	1	12	1	13	10	8	25	3	3	13	10	8	25	3	2	10	<1	1	1	1	8	31	3	10	8	8	8
Other insects		12	5	9	9	3	21	13	48	40	13	13	21	13	48	40	13	13	2	1	5	<1	<1	24	3	9	19	3	3	3
Insect eggs		6	2	<1	15	5	13	8	3	20	13	13	13	8	3	20	13	2	2	<1	<1	<1	<1	3	3	3	3	3	3	3
Cladocera				1					3						3															
Araneae					4						5	5					5													
Hydracarina		11	<1	3	1	1	28	5	20	10	10	10	<1	1	<1	<1	2	<1	1	<1	<1	2	2	3	10	3	3	3	3	3
Fish	J	6	24	5	4		8	28	8	3	3	3	8	28	8	3	3													
Detritus		<1	<1	<1	<1	<1	3	3	3	3	3	3	3	3	3	3	3	<1	<1	<1	<1	<1	<1	<1	<1	5	10	85	85	85
OOM		16	28	24	22		15	55	65	30	30	30	15	55	65	30	30	28	76	83	71	84	84	38	92	100	100	100	100	100
Rock		<1	2	2	2	<1	5	18	30	8	8	8	5	18	30	8	8	5	5	5	5	9	9	49	73	70	70	70	70	70
Seed		2	2	1			3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

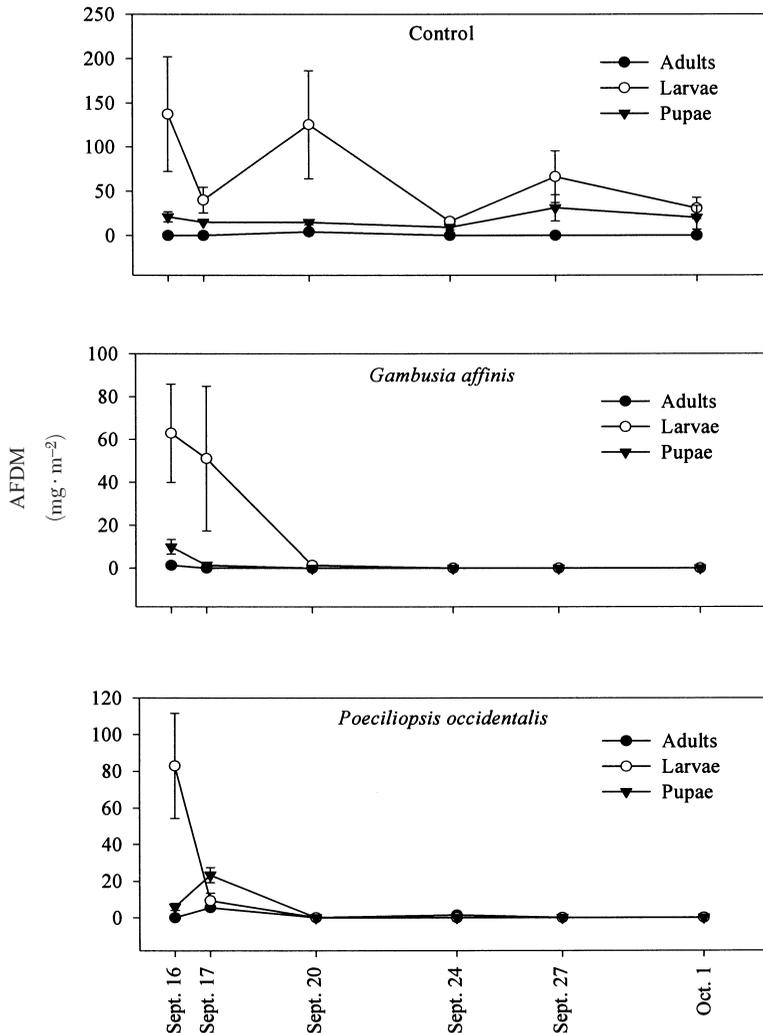


Fig. 1. Ash-free dry mass (AFDM, $\text{mg} \cdot \text{m}^{-2}$) of mosquito life stages available in outdoor control tanks, tanks containing *Gambusia affinis*, and tanks containing *Poeciliopsis occidentalis* during the foraging experiment at Bubbling Ponds Hatchery. Vertical bars represent standard errors of mean biomass.

due to the small magnitudes and the ability of both species to acclimate to high water temperatures over time (Heath 1962, Hagen 1964). The primary behavioral difference is the piscivorous nature of *G. affinis*, a trait which has implicated this nonnative fish in the rapid decline of not only *P. occidentalis*, but also many other native fish species in the Gila River basin and around the globe.

Differences between *P. occidentalis* and *G. affinis* in acute tolerance to un-ionized ammonia indicate *G. affinis* could exist in some habitats where *P. occidentalis* would be excluded. However, recent studies (King et al. 1999, Boyle

and Fraleigh 2003) indicate that even effluent-dominated habitats that do not exceed acute median lethal concentrations of un-ionized ammonia for either species may possess un-ionized ammonia levels that exceed ambient chronic water-quality criteria for ammonia (USEPA 1999). This suggests that neither *P. occidentalis* nor *G. affinis* could survive long-term in effluent-dominated reaches.

Finally, no scientific evidence exists to indicate that nonnative *G. affinis* is better-suited than *P. occidentalis* for mosquito abatement in the Gila River basin. Importation and use of *G. affinis* for mosquito control was an arbitrary

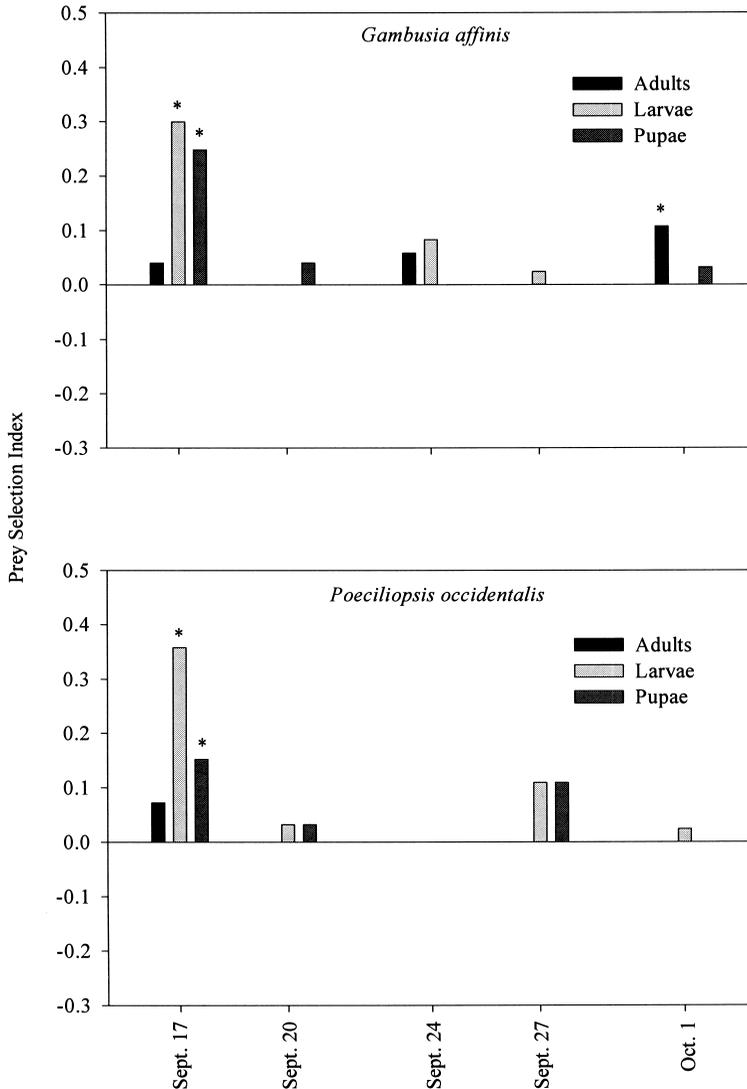


Fig. 2. Prey selection indices by *Gambusia affinis* and *Poeciliopsis occidentalis* for mosquito life stages during the foraging experiment. Vertical bars with asterisks indicate significant selection (Pearre 1982; Yates' chi-square) corrected for multiple tests (Holm 1979). Although *G. affinis* exhibited positive selection for mosquito adults on 1 October 2000, this diet item represented <1% of the total AFDM available on that date. This finding is therefore likely a sampling artifact.

management decision made decades ago without consideration of native fish species for this purpose. As such, *P. occidentalis* should be considered a strong candidate to replace *G. affinis* as a biological control agent of mosquitoes within the native species' former range.

However, managers who would use *P. occidentalis* for mosquito control must be careful not to undo past conservation efforts by contaminating existing lineages (Parker et al. 1999, Hedrick et al. 2001). Choice of source popula-

tions of *P. occidentalis* for mosquito abatement will have to be made on a case-by-case basis to ensure the genetic integrity of existing populations.

It is worth noting that the Arizona Game and Fish Department and the U.S. Fish and Wildlife Service have been working on a range-wide Safe Harbor Agreement for *P. occidentalis* and desert pupfish, *Cyprinodon macularius*. The Safe Harbor Agreement, when finalized and signed by the 2 agency directors, will

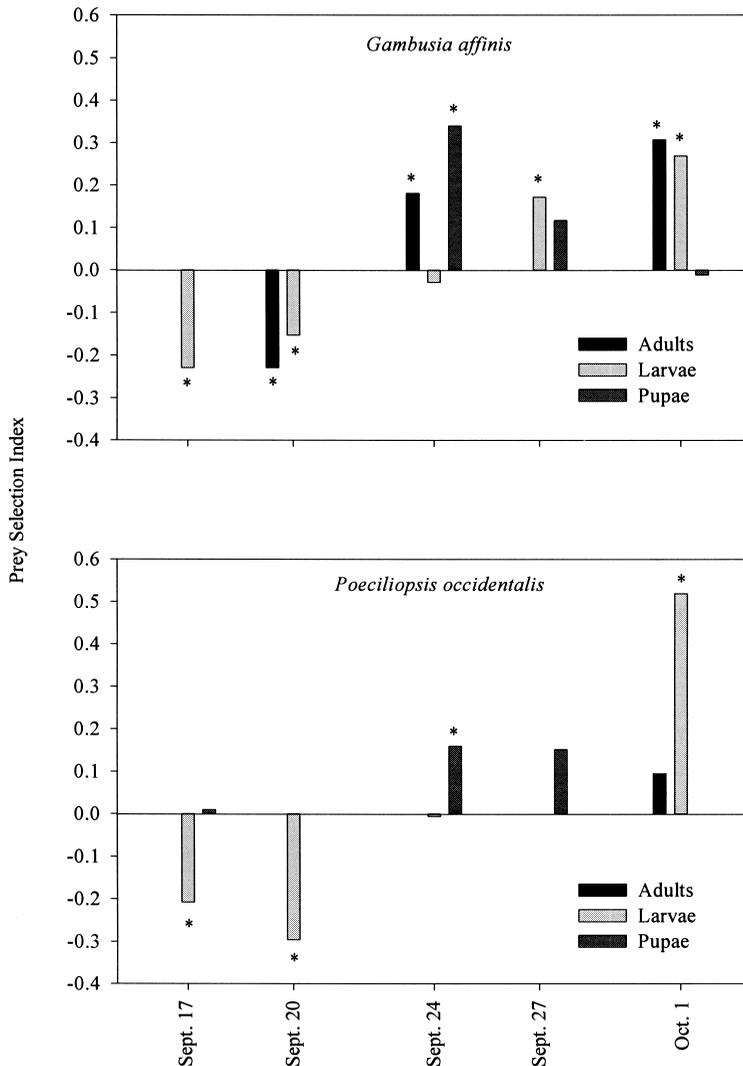


Fig. 3. Prey selection indices by *Gambusia affinis* and *Poeciliopsis occidentalis* for chironomid life stages during the foraging experiment. Vertical bars with asterisks indicate significant selection (Pearre 1982; Yates chi-square) corrected for multiple tests (Holm 1979).

facilitate use of *P. occidentalis* and possibly *C. macularius* for vector control programs. Prior to finalizing the Safe Harbor Agreement, the 2 agencies are completing final revisions of the Gila Topminnow Recovery Plan.

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