Effects of an alien fish, *Gambusia affinis*, on an endemic California fairy shrimp, *Linderiella occidentalis*: implications for conservation of diversity in fishless waters

Karen E. Leyse *, Sharon P. Lawler, Terry Strange [1,2]

Department of Entomology, University of California, Davis, CA 95616-8584, USA

Abstract

The introduction of exotic fishes to naturally fishless waters is often associated with declines in species native to those waters. Mosquitofish, *Gambusia affinis*, have been introduced to waters worldwide for mosquito control. Taxa negatively affected by mosquitofish potentially include fairy shrimp (Crustacea: Anostraca) that often show high rates of endemism, and which are characteristic of naturally fishless wetlands on every continent. We tested the effects of mosquitofish on a fairy shrimp, *Linderiella occidentalis*, in experimental ponds that included a community of native aquatic invertebrates. We supplemented field experiments with lab trials to test whether mosquitofish preferred *L. occidentalis* to other prey. Survival of the fairy shrimp was significantly reduced in ponds with mosquitofish. Only 16.9% of fairy shrimp survived in ponds with mosquitofish, while 64.0% survived in control ponds. Fish presence also reduced abundance and biomass of other invertebrates. Results from feeding trials demonstrated that mosquitofish frequently prefer *L. occidentalis* to alternative prey. Our results suggest that introduction of mosquitofish into naturally fishless wetlands may lead to loss of diversity of their highly specialized aquatic fauna.
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1. Introduction

Biological invasions have long been recognized as a threat to the biodiversity of freshwater systems (Elton, 1958). The effects of non-indigenous introductions on riverine and large lake systems are well documented (Kolar and Lodge, 2000); however, temporary and permanent wetlands that are either remote (mountain lakes and pools) or geographically isolated from river systems (including playa lakes, woodland pools, prairie potholes, and vernal pools) have not been as well studied (Bahls, 1992; King et al., 1996; Tiner et al., 2002). Changes in the biota of these systems may go unnoticed (Mura, 2001). These waters are most often naturally fishless (Stoddard, 1987; Bahls, 1992; Tiner et al., 2002). Worldwide, such geographically isolated wetlands provide habitat for specialized native crustaceans, including over 250 species of Anostraca, the fairy shrimps (Brtek and Mura, 2000). These wetlands are extremely important to amphibians and to migratory birds that rely on the abundant macroinvertebrates for nourishment (see review in Tiner et al., 2002).

Exotic fishes have been intentionally introduced throughout the world for sport fishing, for ornamental purposes, and as biological control agents (Taylor et al., 1984). Introduced fish may spread to additional waters and have been known to eliminate or greatly reduce native fauna through competition or predation (Moyle et al., 1986). Despite biodiversity concerns, however, intentional fish stocking is still increasing globally (Kolar and Lodge, 2000). In naturally fishless waters, the effects of non-indigenous fish may be especially pronounced and such introductions have been correlated with declines of a variety of invertebrates and

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* Corresponding author. Tel./Fax: +1-530-752-1537.
E-mail address: keleyse@ucdavis.edu (K.E. Leyse).
[1] San Joaquin County Mosquito and Vector Abatement District 7759 S. Airport Way, Stockton, CA 95206.
[2] Strange Aquatic Resources, Wilseyville, CA 95257.

amphibians indigenous to those systems (Stoddard, 1987; Fisher and Shaffer, 1996; Gamradt and Kats, 1996; Parker et al., 1996; Englund, 1999; Knapp and Matthews, 2000; Hamer et al., 2002). Although the impacts of fish on natural systems often seem apparent in surveys, correlative field studies do not establish a causal relationship for population declines. Stronger evidence is usually needed to convince policy makers and resource managers that fish introductions are responsible for losses of native species. We present here a replicated experiment designed to quantify effects of a widely introduced fish on a species of fairy shrimp, which are typical of many fishless wetlands worldwide.

The western mosquitofish, *Gambusia affinis*, is a small fish native to the southeastern United States. In the past century this species, and its congener, the eastern mosquitofish, *Gambusia holbrooki*, have been stocked in both permanent and temporary waters throughout the world for mosquito control (Duryea et al., 1996; Swanson et al., 1996). Mosquitofish have a variety of biological attributes typical of successful invaders and have expanded their range to include a diversity of habitats worldwide (Courtney and Meffe, 1989; Englund, 1999; Hamer et al., 2002). Mosquitofish are opportunistic omnivores; in addition to preying on mosquito larvae, their diet includes algae, crustaceans, insects, and vertebrates, including amphibian larvae and small fishes (see review in Swanson et al., 1996). Mosquitofish attack most potential prey encountered; prey preference may be due to a variety of factors including fish size and the size, abundance and activity of potential prey (Linden and Cech, 1990; Swanson et al., 1996). Introduced mosquitofish can substantially reduce zooplankton populations and shift size frequencies of remaining populations through selective predation on larger individuals (Hurlbert and Mulla, 1981). Although such results provide empirical evidence of zooplankton vulnerability to mosquitofish, no one has shown whether mosquitofish reduce abundance of anostracans when zooplankton and other invertebrates are present.

The order Anostraca is one of four primitive orders of phyllopodous branchiopods that are found only in continental waters (Dodson and Frey, 2001; Smith, 2001). Due to the large size of most species, anostracans are among the most conspicuous of the specialized invertebrate fauna characteristic of fishless wetlands (Banarescu, 1990; Eng et al., 1990; King et al., 1996; Simovich, 1998). Anostracans occur on every continent and many oceanic islands (Belk and Brtek, 1995; Brtek and Mura, 2000). At present 27 genera are known to inhabit temporary pools, and seasonally fluctuating, permanent lakes and ponds (Belk, 1981; Belk and Brtek, 1995; Brtek and Mura, 2000). They are found in such varied habitats as astatic waters in river floodplains, rice fields, rock and prairie pools, and deep mountain lakes (Eder et al., 1997; Brtek and Mura, 2000; Mura and

Takami, 2000; Mura, 2001; Smith, 2001). Some species are often extremely abundant in waters where found, while other species are typically rare (Eng et al., 1990).

Species also exhibit clear differences in timing of seasonal occurrence (Eder et al., 1997). They are rarely present in waters with carnivorous fishes (Kerfoot and Lynch, 1987; Smith, 2001). Genera frequently exhibit a substantial degree of speciation and endemism at the local and regional level (Keeley and Zedler, 1998; Smith, 2001). In the past, documentation of branchiopod distributions has been fragmentary in much of the world, but surveys of the fishless wetlands in which they are found have greatly increased in recent years (Eder et al., 1997; Mura and Takami, 2000; Naganawa and Orgiljanova, 2000). A survey of publications listed in the BIOSIS database shows the description of 4 new genera and 12 new species in the last 10 years (Fugate, 1993; Thiéry and Fugate, 1994; Vekhoff, 1998; Belk and Fugate, 2000; Brtek and Mura, 2000; Naganawa and Orgiljanova, 2000; Sanoamuang et al., 2000; Rogers and Fugate, 2001; Timms, 2001; Naganawa and Zagas, 2002; Obregón-Barboza et al., 2002).

We tested the effect of the mosquitofish on a fairy shrimp, the California linderiella, *Linderiella occidentalis*, in a replicated field study using six experimental ponds. *L. occidentalis* is one of nine anostracan species endemic to California vernal pools (Eriksen and Belk, 1999). *L. occidentalis* frequently co-occurs in pool complexes with three fairy shrimp protected under the Endangered Species Act: *Branchinecta lynchi* (threatened), *B. conservatio* (endangered), and *B. longiantenna* (endangered) (Eng et al., 1990; Federal Register, 1994; Helm, 1998; Eriksen and Belk, 1999). *L. occidentalis* is similar in behavior to these and other anostracans, and so may serve as a suitable indicator of *G. affinis* effects on other fairy shrimp species.

Fairy shrimp and other phyllopods are not considered important in the diets of fish because they generally occur in bodies of water that do not naturally support fish (Smith, 2001). Likewise, fish predation has seldom been considered a factor in their persistence (Eng et al., 1990; King et al., 1996; but see Federal Register, 1994). Fishless waters including vernal pools, however, have been considered potential mosquito breeding sources and possible public health hazards (Bohart and Washino, 1978; Wright, 1991; Federal Register, 1994; Duryea et al., 1996). In the United States, mosquito control practices have included stocking mosquitofish in fishless wetlands, including vernal and woodland pools (Federal Register, 1994; Duryea et al., 1996). Fish may also be washed into these wetlands from permanent waters during floods (Smith, 2001).

Mosquitofish have been suggested as possible factors in the declines of vernal pool fauna in California (Shaffer et al., 1993) where surveys suggested an inverse relationship between presence of vernal pool species and

introduced vertebrate predators, including mosquitofish. However, because the various introduced predators often co-occurred in ponds, the authors were not able to calculate an unconfounded correlation between mosquitofish and vernal pool species. The survey results indicated a need to complete manipulative experiments to test whether mosquitofish presence alone might lead to changes in species diversity in vernal pool communities.

To our knowledge mosquitofish effects on fairy shrimp have not been quantified in a controlled and replicated experiment. We were asked to help resolve questions about the effect of mosquitofish on native vernal pools species (personal communication, Peter Ghormley, North Salinas Valley Mosquito Abatement District). In addition to testing whether mosquitofish reduced fairy shrimp survival, we also tested their effects on the total invertebrate community. We supplemented the field experiment with laboratory experiments to help identify an explanatory mechanism for field results.

2. Methods

2.1. Field experiment

We conducted the field study in six 3.05 m × 6.1 m rectangular ponds located at the White Slough Mosquitofish Rearing Facility near Lodi, San Joaquin County, California. The ponds were arranged in one row of six ponds. To intersperse treatments (Hurlbert, 1984) we alternated treatment and control ponds after randomly selecting the treatment for pond 1. We lined each pond with polyethylene sheeting and placed soil over the lining. Ponds sloped gradually at one end to a maximum depth of approximately 60 cm. Each pond was surrounded by a fence of aluminum flashing that extended approximately 20 cm below the soil surface and was 30 cm high. The flashing served as a deterrent to non-native crayfish and small terrestrial animals; however, the ponds were open to large terrestrial vertebrates, to birds, and to colonization by flying aquatic insects. In December 1997, the ponds were filled with untreated well water. Frequent rains and a back up float-valve irrigation system maintained water levels throughout the experiment. Pond basins lacked vegetation and remained turbid for the duration of the experiment.

On December 30, we inoculated all experimental ponds with 1.2 L of concentrated pond water containing zooplankton from roadside ditches and temporary pools in vernal pool habitat. We collected zooplankton from locations without fairy shrimp. We added additional inoculations of concentrated temporary pool zooplankton on January 24 (1.6 L) and January 30 (1.2 L). On each date, collections from source ponds were pooled before being equally distributed among all experimental ponds.

We collected *L. occidentalis* from roadside ditches and vernal pools in southern Sacramento and San Joaquin Counties, California. We obtained mosquitofish from fish rearing ponds at the mosquitofish rearing facility. On January 24, 1998, we stocked each experimental pond with 300 *L. occidentalis* (total length: mean = 9.64 mm, SD = 0.44). We added 12 adult mosquitofish, including at least 2 males, (total length: mean = 34.33 mm, SD = 3.78) to each of three (treatment) ponds. Our use of 12 fish is within the density range that might be added to pools where fairy shrimp occur. California mosquito control districts often stock mosquitofish at a rate of 0.56–1.68 kg/ha in lakes, streams and marshes (Downs, 1991), which would provide an experimental pond density of 7–22 fish.

We added 250 additional *L. occidentalis* (total length: mean = 6.82 mm, SD = 1.61) to each pond on January 31, and a final 50 *L. occidentalis* on February 12. *L. occidentalis* in our ponds had a maximum density of 129/m^3. Densities of fairy shrimp in natural pools vary widely; surveys of vernal pools with *L. occidentalis* provide estimated densities of 9–300,000/m^3 (King, Simovich and Brusca, unpublished data). Anostraca have an annual life cycle and many species, including *L. occidentalis*, produce only resting eggs, or cysts, that hatch when pool substrates are re-hydrated with the return of seasonal rains (Smith, 2001), so successive additions of fairy shrimp simulated natural variation in egg hatch that occurs as pools fill.

On March 3, we seined each pond 3 times with a 3.2 mm mesh seine to count the *L. occidentalis* before they began to senesce. We also captured and counted surviving fish. We did not detect any mosquitofish recruitment during the period of the experiment, and all but 2 adult fish survived.

On March 5, we sampled ponds for aquatic insects using a rectangular handnet frame (24.5 cm × 17.5 cm) fitted with 0.35 mm mesh silk netting. We drew the net along the subsurface for 1 m so that the top 1 cm (approx.) of substrate was sampled, along with the overlying water. We collected a sweep from each of three sides of each pond, pooled the sweep contents for each pond, placed them in 70% alcohol, and transported them to the lab where the insects in each sample were counted. We recorded insect biomass after drying insects in an oven at 55 °C until at stable weight (measured to 0.0001 g).

We collected zooplankton samples with the same net used for insects. We made five 1 m draws through the water, two at and three below the water surface. Each tow was drawn from a different area of the pond and these areas were consistent for all ponds. Zooplankton tow samples were pooled for each pond. In the lab we standardized each zooplankton sample volume at 50 ml,

then counted zooplankton ($\geqslant$1/2 mm total length) in five 1.0 ml subsamples to determine averages per unit volume for analysis.

We performed an arcsin-square-root transformation on the proportion of *L. occidentalis* surviving, then used analysis of variance (ANOVA) to test the primary hypothesis that mosquitofish affected fairy shrimp survival (Scheiner, 1993). We also compared data for other invertebrates from treatment and control ponds. We log transformed the invertebrate data (total insect numbers, total insect biomass, and total zooplankton numbers) then performed a MANOVA to determine mosquitofish effects on these response variables. This analysis protects the experimental error rate while allowing detection of interactions among variables (Scheiner, 1993). We performed analyses with the JMP IN 3.2.6 statistical package (Sall and Lehman, 1996).

2.2. Lab experiments

We conducted feeding trials to test mosquitofish preference for *L. occidentalis* versus alternative prey species (*Daphnia*, mosquito larvae, copepods and corixids). The laboratory experiments were conducted in 20.8 L aquaria that were opaque on three sides and coated on the fourth side with one-way mirrored film. The tanks were equipped with a plastic divider to separate species before testing and were lit from above with 15 W fluorescent lights. The tanks had 1.5 cm deep gravel substrates and were filled with spring water at room temperature. Each tank contained one plastic plant to provide cover.

We collected mosquitofish from White Slough fish rearing ponds, then held them in a 75.7 L aquarium filled with aerated spring water. Fish were fed ad-lib one to two times daily (Tetra GuppyMin tropical flakes). We obtained mosquito larvae from mosquito abatement district facilities. We collected other invertebrates from temporary pools in vernal pool habitat.

We conducted six trials for each prey combination. In each trial, we placed a fish in an aquarium on one side of the divider and 15 *L. occidentalis* plus 15 alternate prey on the other side of the divider. After an acclimation period of one hour, we removed the divider, and the fish were allowed to feed until 30 minutes passed or half the prey were consumed. We recorded the number of attacks and predation events during each trial.

We conducted initial feeding preference trials in 1998. All organisms were housed under ambient light conditions in the lab. Fish were not fed during the 12 h prior to lab experiments. We used only adult female fish that were not visibly pregnant; each fish was used only once. We compared mosquitofish preference for *L. occidentalis* with preference for *Daphnia*, copepods, and mosquito larvae (4th instar *Ochlerotatus dorsalis*). Size measurements for fish and invertebrates are given in Table 1. The total number of prey consumed per trial was low, so in spring 1999 we performed additional trials with fish that were starved longer.

In 1999, all organisms were held under a 12 h light/12 h dark light cycle at 20.5 °C. Fish were not fed for 24 h prior to lab experiments. Set up of experimental aquaria was the same as in 1998. We conducted tests comparing mosquitofish preference for *L. occidentalis* with *Daphnia*, mosquito larvae (4th instar *Culex tarsalis*), and corixids. We compared mosquitofish preference for *L. occidentalis* to preference for corixids because corixids were abundant in vernal pools in the spring of 1999. We were unable to locate sufficient copepods for our 1999 trials. Mean sizes of *L. occidentalis* were smaller than in 1998, while mean sizes of fish and *Daphnia* were larger (Table 1).

For each trial we summed the number of individuals eaten and attacked. We analyzed totals using the χ^2 test for goodness of fit (Sokal and Rohlf, 1995). Two trials in which the fish neither ate nor attacked either prey were excluded from the respective analyses. Total length measurements were log transformed before comparison of lengths by year.

3. Results

3.1. Field experiment

Mosquitofish significantly reduced *L. occidentalis* numbers in experimental ponds relative to control ponds

Table 1
Comparison of mean total lengths (TL) of mosquitofish and invertebrates used in feeding preference trials in 1998 and 1999

Taxon	Year: 1998		Year: 1999		Wilcoxon two-sample test	
	Mean TL	SD	Mean TL	SD	z	P
Mosquitofish	34.00	3.21	37.54	5.32	2.324	0.02
L. occidentalis	10.32	1.29	7.74	0.43	−3.036	0.0024
Daphnia	1.30	0.19	3.95	0.04	3.845	0.0001
O. dorsalis	6.10	0.57				
C. tarsalis			5.77	0.44		
Copepods	4.27	0.35				
Corixids			3.52	0.74		

Lengths are in millimeters. We used a non-parametric test because unequal numbers of subjects were measured between the years.

Table 2
MANOVA for the effects of mosquitofish on the invertebrate pond community: insect biomass, and insect and zooplankton abundance

Source of variation	df	Wilks' lambda	SS	F	P
(a) MANOVA					
Treatment	1, 4	0.21799	...	14.349	0.0193
(b) ANOVA					
Insect biomass	1, 4	...	1.696123	11.018	0.0294
Insect abundance	1, 4	...	0.893102	4.417	0.1035
Zooplankton abundance	1, 4	...	0.163350	1.682	0.2644

Individual ANOVAs are given only to indicate the relative contribution of each variable to the treatment effect.

($F_{1,4} = 12.13, P = 0.0253$). In the control ponds, 64% (SD = 11.73%) of the fairy shrimp survived, while only 16.9% (SD = 10.86%) survived in ponds with fish.

Mosquitofish presence altered pond community structure (statistics are presented in Table 2). The mean biomass of aquatic insects sampled from ponds with fish was 11% of the biomass from control ponds (control mean = 0.0188 g, SD = 0.0089; fish mean = 0.0020 g, SD = 0.0015). Fish reduced the mean abundance of aquatic insects (Chironomidae, Corixidae, Coleoptera and Notonectidae) in samples by 87% (control mean = 160.00, SD = 174.85; fish mean = 20.33, SD = 13.01). Chironomids comprised 85% of the insects collected in substrate samples and total insect abundance reflected the treatment difference in their numbers. Mean abundance of zooplankton in samples from fish ponds was 88.87 (SD = 93.27) while in control pond samples it was 132.60 (SD = 16.92).

3.2. Lab experiment

Feeding trials indicated that mosquitofish readily feed on *L. occidentalis*; however preference results were mixed. In the 1998 trials, mosquitofish consumed more *L. occidentalis* than *Daphnia* ($X^2_{(1)}$ for goodness of fit = 7.538, $P < 0.01$, Fig. 1A), but consumed more copepods than *L. occidentalis* ($X^2_{(1)} = 10.756$, $P < 0.005$, Fig. 1C). We did not detect a difference in mosquitofish consumption of *L. occidentalis* and *O. dorsalis* ($X^2_{(1)} = 1.316$, n.s., Fig. 1B). Total attacks (prey either eaten or rejected) by mosquitofish indicated no detectable difference between number of attacks on *L. occidentalis* versus *Daphnia* ($X^2_{(1)} = 0.010$, n.s., Fig. 1A) and *L. occidentalis* versus *O. dorsalis* ($X^2_{(1)} = 3.5957$, n.s., Fig. 1B). The total number of mosquitofish attacks on copepods was significantly higher than the attacks on *L. occidentalis* ($X^2_{(1)} = 73.8409$, $P < 0.001$, Fig. 1C).

In the 1999 feeding trials, mosquitofish consumed *L. occidentalis* in preference to alternative prey in all three trials (*L. occidentalis* versus *C. tarsalis*: $X^2_{(1)}$ for goodness of fit = 27.94, $P < 0.001$, Fig. 1E; *L. occidentalis* versus *Daphnia*: $X^2_{(1)} = 25.47$, $P < 0.001$, Fig. 1D; *L. occidentalis* versus corixids: $X^2_{(1)} = 36.75$, $P < 0.001$, Fig. 1F). A comparison of the total number of attacks by mosqui-

tofish indicated that mosquitofish attacked *L. occidentalis* more often than either mosquito larvae ($X^2_{(1)} = 25.09$, $P < 0.001$, Fig. 1E) or corixids ($X^2_{(1)} = 25.14$, $P = < 0.001$, Fig. 1F), but attacked *Daphnia* and *L. occidentalis* at indistinguishable rates ($X^2_{(1)} = 2.52$, n.s., Fig. 1D). Fish often attacked *Daphnia* multiple times, then rejected the prey.

4. Discussion

Our results provide empirical evidence that mosquitofish can significantly reduce *L. occidentalis* abundance in pool communities. The combination of field and lab results strongly suggests that predation is the mechanism by which fairy shrimp survivorship was reduced in treatment ponds. Results from feeding trials demonstrate that mosquitofish feed readily on *L. occidentalis* when there are alternative prey available, but that mosquitofish prey preference might be altered by the size relationships of available prey and starvation levels of fish. Although we cannot rule out the possibility that there is simply a year effect in differences in prey preference between 1998 and 1999, our interpretation is consistent with work by Bence and Murdoch (1986) that indicated that mosquitofish prey preference was affected by fish size in relation to prey size and fish levels of satiation. These lab results are also consistent with the overall effect of mosquitofish on other experimental pond invertebrates. Our ponds were stocked with zooplankton from shallow pools and colonized by insect taxa all commonly found co-occurring with anostracans. A MANOVA indicated that mosquitofish affected the structure of this community. The loss of insect biomass between the control and treatment ponds was of greater magnitude than the downward trend in insect numbers, suggesting that mosquitofish were preying preferentially on larger individuals.

Because mosquitofish are known to be visual predators (Swanson et al., 1996), the magnitude of the reduction in *L. occidentalis* abundance was surprising given the low number of fish in each pond and the turbid condition of the ponds during the experiment. Competition is possible, but we think this is an unlikely
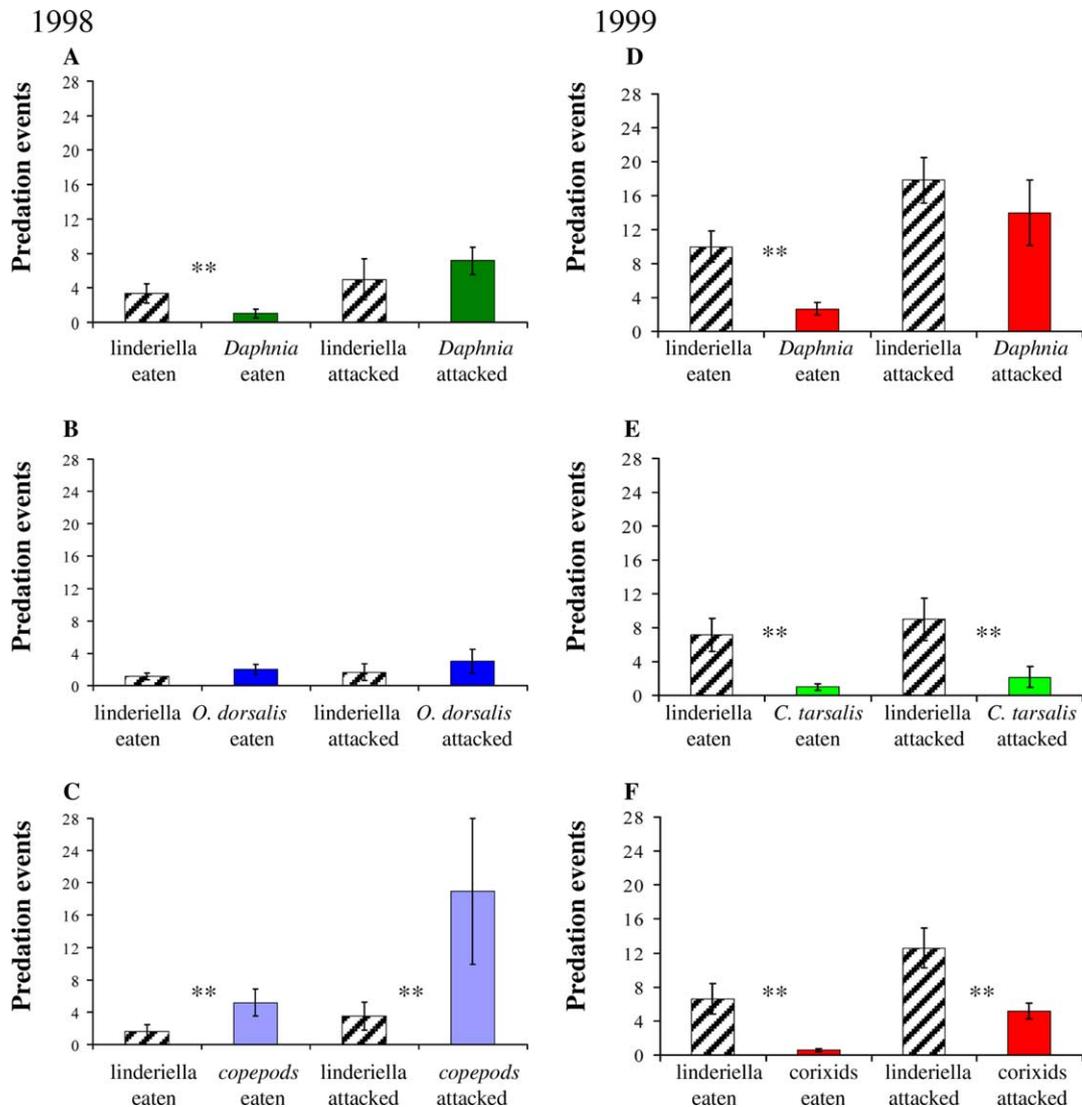
Fig. 1. Feeding preferences of mosquitofish for the fairy shrimp, *L. occidentalis*, in comparison to co-occurring invertebrates. *L. occidentalis* are referred to as linderiella in the graphs. The bars show the mean number of each prey eaten by one mosquitofish for each prey comparison. A–C are 1998 tests, while D–F are for 1999 tests. Both predation (only) and total attacks (prey either eaten or rejected) are shown. Error bars designate ±1 standard error. Significant results are designated by asterisks.

mechanism for reduction in *L. occidentalis* abundance because these fairy shrimp feed primarily on bacteria, bits of detritus, algae, and rotifers while mosquitofish typically take the largest prey they are capable of consuming (Swanson et al., 1996; Smith, 2001) and all fish present were adults.

Our earthen experimental ponds minimized the variation in pond size and depth that we would encounter if we used natural pools. We were able to standardize not only predator densities, but densities of focal prey, giving us more statistical power. These model systems allow a level of control and rigor that is generally not possible in natural systems (Resetarits and Fauth, 1998). The realism of experiments conducted in artificial ponds has been challenged in the past, primarily as a result of high densities of subject taxa used in the experiments (see review in Resetarits and Fauth, 1998). However, Resetarits and Fauth (1998) found that mesocosm experiments could, when properly grounded in natural history, successfully make specific predictions about the structure and dynamics of natural pool communities.

We attempted to provide realistic conditions in our experiments. *L. occidentalis* occur in widespread locations throughout California's Central Valley. They have been collected from pools ranging in size from 1 m² to 40 ha (Eng et al., 1990). Our experimental ponds were located within the range of *L. occidentalis* and were exposed to the same weather conditions as local vernal pools. Branchiopods generally tolerate wide ranges in temperature and pH, in keeping with fluctuating water levels in natural pools (Smith, 2001). Our ponds were within the depth and temperature ranges in which both

L. occidentalis and mosquitofish have been found in field surveys (Shaffer et al., 1993; Gallagher, 1996; King et al., 1996). Although the pH in our experimental ponds ranged from 8.7 to 9.9 while surveys by Eng et al. (1990) indicated *L. occidentalis* commonly occurred in poorly buffered waters between 6.1 and 8.4 pH, it did not vary by treatment (Student's t test, df $= 4$, $t = 0.878$, $P = 0.429$). *L. occidentalis* occur in mildly tannic pools with grass substrates and in turbid, mud-bottomed pools like those we used. Experimental results could differ in some pools if vegetative structure provides fairy shrimp with refuge from mosquitofish.

Our initial densities of fairy shrimp were lower than the densities of *L. occidentalis* in many natural pools; however, densities were within the range of densities encountered in the field (King, Simovich, and Brusca, unpublished data). In addition, they are representative of densities of other fairy shrimp species observed in a variety of California habitats (Leyse, personal observations). We introduced mosquitofish to our experimental ponds based on standard stocking rates used by mosquito control agencies. Our results may underestimate fish effects because fish may be stocked at higher densities in response to specific situations (Downs, 1991). Higher densities may also occur when mosquitofish are moved into new waters by floods, when pools do not dry completely each year, or when fish breed (see review in Swanson et al., 1996).

We do not mean to suggest that mosquitofish are responsible for all known declines in fairy shrimp abundance. In numerous areas of the world, loss of astatic pool habitat due to land type conversion is recognized as the primary threat to Anostraca species persistence (Eng et al., 1990; Petrov and Petrov, 1997). However, we wish to call attention to a possible threat to species diversity in normally fishless wetlands that may go unnoticed because knowledge about the aquatic invertebrate fauna of these areas is imperfect (Mura, 2001) and many such wetlands are not characterized by the specialized flora typical of California's vernal pools.

In California, past mosquito control practices included stocking mosquitofish in vernal pools (Federal Register, 1994), but a brief survey of "mosquito and vector control district" websites indicates that in recent years some have modified their practices to protect known protected species. However, even where districts have modified their practices, fish are still often available to the public for stocking. Mosquitofish now occur worldwide and some countries may not yet have protected endemic and rare species in fishless waters (Petrov and Petrov, 1997). In some countries, species are in danger of extinction (Maier, 1998; Samraoui, 2002). In addition, it is apparent from the recent literature that the discovery of new species is expanding (e.g., King et al., 1996; Vekhoff, 1998; Belk and Fugate, 2000; Brtek and Mura, 2000; Naganawa and Orgiljanova, 2000;

Sanoamuang et al., 2000; Timms, 2001; Naganawa and Zagas, 2002; Obregón-Barboza et al., 2002). Therefore, we believe it is important to quantify and publish effects of introduced fish on formerly fishless communities.

More work is needed to determine whether measures to control mosquito larvae are needed in such wetlands. In California, surveys have indicated that mosquito larvae are seldom abundant in vernal pools that have not been degraded by humans. Vernal pool surveys conducted by King (Federal Register, 1994) indicated that mosquitoes bred and developed successfully only in pools that were degraded. In non-degraded vernal pools, she only found mosquito larvae late in the season. Wright (1991) determined that patterns of mosquito occurrence in vernal pools suggested that absence or rarity of mosquito larvae was likely due to the characteristic vernal pool invertebrate fauna with its high abundance of predatory crustaceans and aquatic insects. Experimental data have also shown that aquatic predators of fishless waters, such as notostracans, notonectids and odonate nymphs, not only reduce survival of mosquito larvae in pools, but also reduce oviposition by adult females (Fry et al., 1994; Blaustein et al., 1995; Stav et al., 1999; Stav et al., 2000). In some experiments mosquitofish presence has made ponds more suitable for mosquito breeding and resulted in rapid increases in larval mosquitoes (Hoy et al., 1972; Hurlbert and Mulla, 1981).

Given the rarity and endemism of many Anostraca and other crustaceans typical of fishless waters (King et al., 1996; Petrov and Petrov, 1997; Maier, 1998; Samraoui, 2002), a precautionary approach to their conservation would be to complete invertebrate surveys of wetlands (including nearby habitat where dispersal into fishless wetlands is possible) prior to considering mosquitofish introductions. Surveys should be conducted every few weeks while wetlands hold water because different fairy shrimp species hatch at different times. Such surveys could reveal both whether mosquitoes are a problem and whether anostracans are present, leading to more informed management decisions. To complement field surveys, we have provided experimental evidence that presence of mosquitofish in fishless pools may result in significant reductions in anostracan populations. The fact that Anostraca rarely occur in waters with carnivorous fishes (Smith, 2001) indicates that cyst banks do not provide protection against sustained mortality of active fairy shrimp due to fish predation.

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