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Effects of Introduced Mosquitofish and Bullfrogs on the Threatened California Red-Legged Frog

SHARON P. LAWLER,*‡ DEBORAH DRITZ,* TERRY STRANGE,†
AND MARCEL HOLYOAK*

*Department of Entomology, University of California, Davis, CA 95616–8584, U.S.A.
†San Joaquin County Mosquito and Vector Control District, 7759 South Airport Way, Stockton, CA 95206, U.S.A.

Abstract: *Exotic species have frequently caused declines of native fauna and may contribute to some cases of amphibian decline. Introductions of mosquitofish (*Gambusia affinis*) and bullfrogs (*Rana catesbeiana*) are suspected to have caused the decline of California red-legged frogs (*Rana aurora draytonii*). We tested the effects of mosquitofish and bullfrog tadpoles on red-legged frog tadpoles in spatially complex, speciose communities. We added 720 hatchling red-legged frog tadpoles to each of 12 earthen ponds. Three ponds were controls, 3 were stocked with 50 bullfrog tadpoles, 3 with 8 adult mosquitofish, and 3 with 50 bullfrogs plus 8 mosquitofish. We performed tests in aquaria to determine whether red-legged frog tadpoles are preferred prey of mosquitofish. Mosquitofish fed on a mixture of equal numbers of tadpoles and either mosquitoes, Daphnia, or corixids until < 50% of prey were eaten; then we calculated whether there was disproportionate predation on tadpoles. We also recorded the activity of tadpoles in the presence and absence of mosquitofish to test whether mosquitofish interfere with tadpole foraging. Survival of red-legged frogs in the presence of bullfrog tadpoles was less than 5%; survival was 34% in control ponds. Mosquitofish did not affect red-legged frog survival, even though fish became abundant (approximately 1011 per pond). Two mechanisms may have blocked the effects of mosquitofish on tadpole survival: (1) fish ponds contained fewer predatory invertebrates, and (2) mosquitofish preferred other prey to red-legged frogs in laboratory trials. Red-legged frog tadpoles suffered more injuries in ponds with fish, however, and weighed 34% less at metamorphosis. The growth decrease could have been caused by injuries or by lower foraging levels in the presence of fish. Laboratory results showed that young tadpoles were less active in the presence of mosquitofish. Although both mosquitofish and bullfrogs affected red-legged frogs, the impact of bullfrogs on the survival of red-legged frogs may contribute more strongly to their decline.*

Effectos de la Introducción del Pez Mosquito y Rana Toro en Poblaciones de la Rana

Resumen: *Especies exóticas han ocasionado frecuentemente disminuciones de fauna nativa y pueden contribuir en algunos casos a la disminución de anfibios. Introducciones del pez mosquito (*Gambusia affinis*) y rana toro (*Rana catesbeiana*) son consideradas como la causa del declive de la rana patiroja de California (*Rana aurora draytonii*). Evaluamos los efectos del pez mosquito y renacuajos de rana toro en renacuajos de rana patiroja en comunidades espacialmente complejas. Agregamos 720 renacuajos recién eclosionados a cada uno de los 12 estanques de tierra. Tres de los estanques eran controles, tres fueron sembrados con 50 renacuajos de rana toro, tres con 8 adultos de pez mosquito y 3 con 50 renacuajos de rana toro mas ocho peces mosquito. Realizamos pruebas en acuarios para checar si los renacuajos de patiroja eran la presa preferida del pez mosquito. Los peces mosquito se alimentaron de una mezcla de igual número de renacuajos y tanto mosquitos, Daphnias, o coríxidos, evaluamos si hubo una depredación desproporcionada de renacuajos una vez que alrededor del 50% de las presas fué comido. También observamos la actividad de los renacuajos en presencia y ausencia de peces mosquito para determinar si los peces interferían con el forrajeo de los renacuajos. La supervivencia de ranas patiroja en presencia de renacuajos de rana toro fue redu-*

cida en un 5%; la supervivencia en los estanques control fué de un 34%. El pez mosquito no afectó la supervivencia de la rana patiroja, aún cuando la abundancia de peces se tornó elevada (~1011 por estanque). Dos mecanismos pueden haber bloqueado los efectos del pez mosquito en la supervivencia de renacuajos: (1) los estanques con peces tuvieron menos invertebrados depredadores y (2) los peces mosquito prefirieron otro tipo de presas en los experimentos de laboratorio. Sin embargo, los renacuajos de rana patiroja sufrieron mas lesiones en estanques con peces y pesaron 34% menos durante metamorfósis. La disminución en crecimiento pudo haber sido causada por lesines o por niveles mas bajos de forrajeo en presencia de peces. Los resultados de laboratorio muestran que los renacuajo jóvenes fueron menos activos en presencia de peces mosquito. A pesar de que tanto los peces mosquito como la rana toro afectaron a la rana patiroja, el impacto de la rana toro en la supervivencia de la rana patiroja contribuye mas fuertemente a su disminución.

Introduction

Introduced species have caused declines or extinctions of native species worldwide. Some of the most ecologically destructive introductions have been those in which the alien species is a vertebrate that was introduced for economic reasons (Williamson 1996) or when a generalist predator is introduced as a biological control agent (Howarth 1991; Simberloff & Stiling 1996). Species are rarely introduced singly or in the absence of other changes, such as habitat destruction, and this can make it difficult to relate the decline of a native species to the introduction of a particular alien species (Williamson 1996). Experimental work is necessary to estimate the effects of any one factor, preferably by manipulating one or more species independently under conditions that are as natural as possible. We present a field experiment that quantified the effects of two introduced vertebrate species on tadpoles of an endangered frog.

Non-native vertebrate species are suspected to have contributed to the decline of the California red-legged frog (*Rana aurora draytonii*), which is listed as a threatened species. The decline of this formerly abundant species is one of the most dramatic examples of amphibian decline (A. R. Blaustein et al. 1994), and examining causes for this phenomenon could provide clues that may to aid in the recovery of other declining amphibians. Historically, *R. a. draytonii* bred throughout the lower central valley of California and in low elevations in the Sierra and coastal ranges, but it is now restricted largely to the coastal foothills (Jennings & Hayes 1985; Fisher & Shaffer 1996). A dramatic early decline began in 1895 and was associated with human consumption of frogs, but the continued loss of populations is poorly understood (Jennings & Hayes 1985). Possible mechanisms include habitat loss or degradation, competition with and predation from the bullfrog (*Rana catesbeiana*), and predation by introduced fishes (Hayes & Jennings 1986). Some researchers have concluded that introduced predators are the most likely cause of red-legged frog disappearance, although multiple factors probably contribute to the decline (Hayes & Jennings 1986; Fisher & Shaffer 1996).

In a field survey of sites where red-legged frogs have bred historically, Hayes and Jennings (1986) found a strong negative correlation between extant populations of the frog and the presence of introduced fish and bullfrogs. We measured how tadpoles of red-legged frogs are affected by bullfrogs and mosquitofish (*Gambusia affinis affinis*). We selected the mosquitofish because of its widespread and ongoing introduction (Rupp 1996); other species of fish may also play a role in the decline of red-legged frogs.

Bullfrogs are native to the eastern United States. They were introduced in the west to improve the frog "fishery" and have been introduced to Europe as well (Stumpel 1992). Bullfrog tadpoles can be strong competitors (Werner 1994; Kupferberg 1995). Red-legged frog tadpoles face competition from large, overwintering bullfrog tadpoles because bullfrogs breed in summer and red-legged frogs breed from January to March. Larger tadpoles sometimes enjoy a greater per-capita competitive advantage (Lawler & Morin 1993; Werner 1994; Kupferberg 1995; Peacor & Werner 1997). In addition, bullfrog tadpoles may consume northern red-legged frog tadpoles (*Rana aurora aurora*) under some circumstances (Kiesecker & Blaustein 1997). The effects of bullfrog adults on native frogs may be even more pronounced because bullfrogs will consume other frogs. Experimental studies are needed to determine the effect of bullfrogs on other amphibians because several studies have documented the decline of native species after bullfrog introductions (Kiesecker & Blaustein 1997).

Mosquitofish are native to the eastern United States, and have been introduced to wetlands worldwide as biological control agents for mosquito larvae. The practice of stocking mosquitofish concerns conservationists because introduced mosquitofish can harm amphibians (e.g., Woodward 1983; Gamradt & Kats 1996) and other native species (Courtenay & Meffe 1989; Rupp 1996). Mosquitofish are capable of eliminating red-legged frog tadpoles from simple communities in small artificial pools even when tadpoles are as large or larger than the mosquitofish (R. Schmeider & R. Nauman, personal communication). Mosquitofish can also injure or kill fish larger

than themselves (Courtenay & Meffe 1989), so it is unlikely that tadpoles could outgrow mosquitofish predation.

The evidence that mosquitofish may play a role in the decline of the red-legged frog is inconclusive because it is based on correlations between species distributions and on predation trials in arenas where habitat and community structure were simplified. Habitat structure can be important to prey persistence (e.g., Crowder & Cooper 1982; Nelson & Bonsdorff 1990; Holyoak & Lawler 1996), and biotic complexity can allow indirect effects that make it difficult to predict whether an observed direct effect of a predator on a prey will translate into altered population dynamics (Wootton 1994; Menge 1995; Polis & Strong 1996). The mosquitofish is a generalist predator that consumes a wide variety of prey (Bence 1988; Linden & Cech 1990; Rupp 1996), including predators that eat tadpoles (e.g., odonates; Smith 1983; Travis et al. 1985) and invertebrates that compete with tadpoles (e.g., mosquitoes; Morin et al. 1988; L. Blaustein & Margalit 1994). The presence of alternative prey might either reduce the impact of the predator on a particular prey (Murdoch 1969) or increase predation if additional prey support a higher abundance of the predator (Holt 1977; Lawler 1993; Holt & Lawton 1994). Predators can sometimes shift the competitive balance between amphibians because tadpoles of some species forage less and grow more slowly in the presence of predators (Woodward 1983; Lawler 1989; Werner 1991).

The crucial question is whether the sum of the direct and indirect effects of the introduced species on red-legged frogs is positive or negative. To address this question we raised red-legged frog tadpoles in replicated, spatially complex ponds that contained the types of alternative prey and other predators commonly found in red-legged frog breeding sites, and we compared the performance of red-legged frog tadpoles in these ponds and in similar ponds with mosquitofish, or bullfrogs, or both. We also collected information on numbers of invertebrate predators and competitors in experimental ponds to assess the likelihood of various indirect effects. To further aid our interpretation of the results of the pond experiment, we conducted laboratory tests on the feeding preferences of mosquitofish and the behavior of tadpoles.

Methods

Pond Study

We constructed 12 earthen ponds in San Joaquin County, California, during the fall of 1995. Each pond was 3.05 × 6.10 m and sloped gradually to 1.22 m deep at one end. Pond bottoms were sealed with clay. We covered approximately 2 m² of the substrate in the shallow end of ponds with cobblestones approximately 10–25 cm in diameter.

Because red-legged frogs typically breed in areas with emergent vegetation, we planted 20 cattails along the southeast corner of each pond and added 100 old cattail leaves to the ponds. An irrigation system with float-valves kept the ponds full of well water. We added a 1-L sample of zooplankton and algae from a nearby wetland to all ponds (concentrated from 20, 2-m plankton net tows), and aquatic insects colonized ponds naturally. We installed aluminum fences 60 cm high around ponds to prevent juvenile frogs from emigrating.

On 15 March 1996 we added 720 newly hatched red-legged frog tadpoles to each pond, which is within the range of natural densities for this species. Hatchlings were obtained from eggs collected in San Joaquin and Contra Costa Counties. We used hatchlings because red-legged frogs lay their eggs in firmly cohesive bunches that would be difficult to separate and count without damaging the developing eggs. Mosquitofish are usually unable to remove embryos from large ranid eggs like red-legged frog eggs (Grubb 1972), but using hatchlings could produce an underestimate of predation if mosquitofish feed at hatching egg masses. Hatchlings from six clutches were mixed before they were counted and added to ponds to minimize variation among replicates due to genetic or parental effects. On the same day, three ponds also received 50 large bullfrog tadpoles (mean total length 10.98 cm ± 1.25 SD), three received eight adult mosquitofish (four males and four pregnant females), three received both bullfrogs and fish, and three controls received no additional vertebrates. Overwintering bullfrog tadpoles were collected from a pond in Stockton, California. Metamorphosing bullfrogs were collected between days 75 and 256. Mean day of metamorphosis was 107 ± 26 SD. A mean of 26 ± 5 SD bullfrogs completed metamorphosis in the ponds, and fish did not affect the number, weight, or date of the metamorphosis of bullfrogs.

The initial number of mosquitofish exceeded the recommended stocking rate of 0.045 kg/acre used by mosquito abatement districts, but it is in keeping with their practice of introducing 6–10 fish to small bodies of water such as cattle watering tanks to ensure that a breeding population will result. Mosquitofish populations are often low early in the year because of natural winter mortality and flushing of fish from pools during winter floods (Meffe 1983; Swanson et al. 1996). Schools of adult and young mosquitofish were visible in all fish ponds within 3 weeks of introduction. On day 124, three observers counted the number of fish >1 cm in total length visible in 25% of the surface of ponds within the top 5 cm. We counted an average of 51 ± 8 SE mosquitofish per pond. Ponds therefore contained an average of 200 fish by that date. This is probably an underestimate because mosquitofish also use deeper waters. We estimated final densities in November (day 249) via mark-release-recapture. We introduced 100 albino mosquitofish

to each pond as marked fish, allowed them to mingle with the normal fish for 1 hour, and then recaptured ≥200 fish to determine the proportion marked. To assess the accuracy of the study we performed a second mark-recapture in three ponds in which we clipped a small section of the tail fin to mark 100 resident fish. We drained these three ponds 1 week later and counted all fish. The study using albinos showed a mean of 1011 ± 139 SE mosquitofish per pond. Analysis of variance (ANOVA) showed that the final census did not differ significantly from the albino study (ANOVA $F = 1.54$, df = 1,4, $p > 0.28$), but the fin-clip method showed a tendency to underestimate abundance compared to the census (ANOVA $F = 5.27$, df = 1,4, $p < 0.09$). Bullfrog tadpoles had no detectable effect on fish abundance (ANOVA $F = 0.45$, df 1,4, $p = 0.535$).

We collected sweep-net samples of invertebrates from all ponds on days 20, 35, 49, 63, 77, 116, and 144. For each sample we took two benthic sweeps and two midwater sweeps of approximately 2 m, with a standard d-ring net (1-mm mesh). These data were analyzed with repeated-measures ANOVA (SYSTAT, 1992). To check whether mosquitofish differentially affected invertebrates that might eat or compete with red-legged frog tadpoles, we divided the data set into two categories for separate repeated-measures ANOVA: "dominant predatory invertebrates" (odonates, belostomatids, coleopterans, and notonectids) and "dominant grazing invertebrates" (ephemeropterans, corixids, chironomids, and gastropods). We further quantified differences in the abundance of predatory insects on 17 July by collecting dragonfly exuviae (cast skins) from pond vegetation and by counting backswimmers visible in 25% of the pond surface.

During the study, we noticed tail injuries in red-legged frog tadpoles. On day 160, we collected a sample of 17 tadpoles from each of two ponds with mosquitofish and 6 tadpoles from each of two without mosquitofish to assess them for tail damage. Bullfrogs were not present in these ponds. Sample sizes differed because fewer tadpoles remained in ponds without mosquitofish.

Red-legged frogs and bullfrogs metamorphosed into juveniles between June and November 1996. We collected the juveniles on 1–3 nights per week. Juveniles were counted and weighed after they resorbed their tails. Red-legged frogs were fed and released in newly established mitigation ponds in Contra Costa County. Bullfrogs were donated to another research project.

We used SYSTAT statistical software (SYSTAT Inc. 1992) to perform ANOVA on red-legged frog survival and weight data. The proportion surviving in each pond was arcsin-square-root-transformed before a fully-factorial ANOVA. Weights of juvenile frogs were ln-transformed before analysis. A fully-factorial ANOVA on red-legged frog weights did not detect the effects of bullfrogs or mosquitofish, but inspection of the data indicated that an effect of mosquitofish had been obscured

by the variable weights of frogs from the mosquitofish-plus-bullfrog treatment. We therefore compared the weights of red-legged frogs from controls to those from the bullfrog-only and mosquitofish-only treatments separately and discarded the bullfrog-plus-mosquitofish data. This gave us more power to detect a fish effect. We used a repeated-measures ANOVA to determine whether fish affected the date of metamorphosis of red-legged frogs, again omitting data from ponds with bullfrogs because few frogs emerged from these ponds and the dates of emergence were quite variable. Because we analyzed three response variables for the red-legged frogs in this experiment, we applied a sequential Bonferroni correction to the alpha levels (Sokal & Rohlf 1995); these are given in the statistical tables.

Laboratory Experiments

Mosquitofish feeding-preference experiments were conducted in 20-L aquaria containing spring water, a layer of gravel 2 cm deep, and a plastic plant. Three sides were covered with paper, and the fourth was covered with one-way mirror film. Tanks were lit from above with a 15-watt fluorescent lamp. Each aquarium was divided with a removable opaque barrier, which allowed fish and their prey to acclimate in different compartments before trials. The barriers were not watertight. Red-legged frog tadpoles were obtained from eggs found in a San Joaquin County pond and held in predator-free ponds. Mosquitofish were provided by local mosquito abatement districts and were fed flake food. All fish were nonpregnant, mature females, and they were not fed for 24 hours preceding trials. Individual organisms were used only once in experiments.

For each trial, an individual fish was placed in an aquarium behind the opaque divider. Fifteen tadpoles and 15 alternative prey were added to the other side of the divider and were allowed to acclimate for 15 minutes. We then removed the divider and observed fish feeding behavior for up to 30 minutes or until 50% of the prey had been consumed. There were five or six trials for each species of alternative prey. For all feeding-preference trials, we analyzed the data using replicated goodness-of-fit tests (Sokal & Rohlf 1995).

We compared attacks by mosquitofish on red-legged frog hatchlings versus mosquito larvae (*Culex tarsalis*) or water boatmen (Corixidae). Hatchling tadpoles measured 1–1.5 cm total length, mosquito larvae were approximately 0.9 cm long, and corixids were approximately 0.7 cm long. We repeated the mosquito trial using larger, more active tadpoles that averaged 4.3 cm total length, developmental stage 26 (Gosner 1960), and included an additional trial using *Daphnia magna* (0.2–0.3 cm long) as the alternative prey. Tadpoles used in these trials were too large for the fish to engulf, but they were still vulnerable to attack.

A related experiment conducted over a 16-hour period tested whether the presence of alternative prey would affect mosquitofish consumption of tadpoles. We placed three fish and three stage-26 tadpoles (similar in size to those of the first feeding-preference trials) in each of eight aquaria. Four aquaria also received 40 late instar *Culex* larvae. We used several fish together in this experiment because mosquitofish often forage in small groups. The experiment lasted from 1800 hours until 1000 hours the next day, at which time prey were counted and checked for injury. Data were analyzed with the general linear model program GLIM (Baker 1987). This program allowed us to assume a binomial distribution of sampling error and to scale the model by mean square error so that changes in deviance were assumed to follow a chi-square distribution. We performed an ANOVA on logit-transformed proportions of tadpoles with tail damage.

To determine whether mosquitofish affect tadpole foraging, we compared the activity level of tadpoles in the presence and absence of fish in two experiments, one when tadpoles were at developmental stage 26 and mean length 2.4 cm and the second when tadpoles were at developmental stage 33–36 and at a mean length of 5.9 cm. The stage-26 tadpoles in this trial were much smaller than those used in the second feeding trial described above, but it is not unusual to find a large range of body sizes in the early developmental stages because tadpoles accomplish much of their growth during these stages. For each trial, a single tadpole and either nothing (control) or a single fish were added to different compartments of an aquarium. Animals acclimated overnight. The next day, the investigator removed the divider and recorded tadpole activity every 15 seconds for 10 minutes. There were eight control trials and eight fish trials for each experiment. Data were analyzed via GLIM with a logit link function of the proportion of observations in which tadpoles were active. Maximum likelihoods were calculated by a fitting procedure in which changes in deviance were assumed to follow a chi-square distribution.

Results

Pond Study

Bullfrog tadpoles decreased the survival of red-legged frogs. Fewer than 5% of red-legged frogs survived in ponds with bullfrog tadpoles, in contrast to 30–40% survival in other ponds (Table 1; Fig. 1). Mosquitofish had no detectable effect on red-legged frog survival, and we could not detect any interaction between the effects of bullfrogs and mosquitofish on red-legged frog survival.

The presence of bullfrogs and mosquitofish delayed red-legged frog metamorphosis. Red-legged frogs began

Table 1. ANOVA of the effects of bullfrog tadpoles and mosquitofish on the proportion of red-legged frog tadpoles that survived to become juvenile frogs.*

Source	df	Mean square	F	p
Bullfrogs	1	0.75	88.18	<0.001
Mosquitofish	1	0.00	0.01	0.898
Bullfrogs × mosquitofish	1	0.00	0.79	0.399
Error	8	0.00		

Data were arcsin-square-root-transformed before analysis. The sequential Bonferroni-corrected alpha level for this test is 0.016.

to emerge from ponds with bullfrogs approximately 20 days after they first emerged from the other ponds (Fig. 1). Although the dates of first emergence were comparable in ponds with red-legged frogs alone and in those with mosquitofish, a repeated-measures ANOVA showed that emergence lagged in ponds with mosquitofish after the first few weeks (Table 2; Fig. 1).

There was no detectable effect of bullfrog tadpoles on the weight of juvenile red-legged frogs (Table 3). The weights of the few red-legged frogs that emerged from ponds with bullfrogs were variable. In contrast, the frogs that emerged from ponds with mosquitofish weighed 34% less than those emerging from control ponds (Table 3; Fig. 2).

At least part of the weight decrease in frogs raised with fish might be explained by injuries inflicted on the tadpoles by mosquitofish. A sample of tadpoles col-
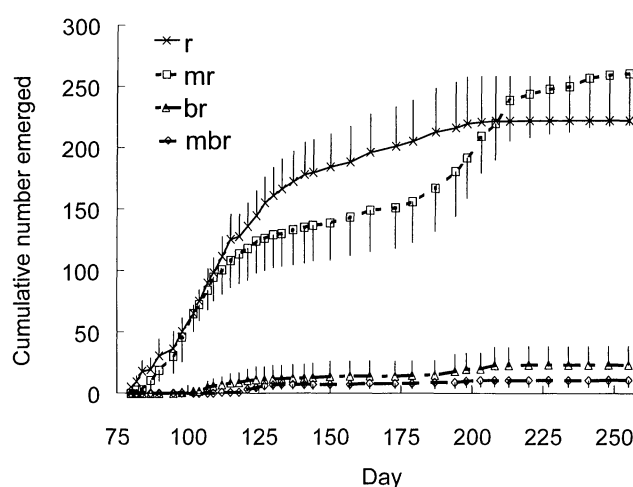
Figure 1. Mean cumulative survival to metamorphosis of 720 red-legged frog tadpoles raised in ponds alone, with mosquitofish, with bullfrog tadpoles, and with both mosquitofish and bullfrog tadpoles (r, ponds with red-legged frogs alone; mr, ponds with mosquitofish; br, ponds with bullfrogs; and mbr, ponds with mosquitofish and bullfrogs). Error bars are standard deviations.

Table 2.   Results of a repeated-measures ANOVA on the effects of mosquitofish (fish) on the number of red-legged frogs emerging from ponds on 39 collection dates.*

Source	df	Mean square	F	p
Between subjects				
Fish	1	88.61	0.65	0.463
Within subjects				
Day	38	86.69	2.42	0.001
Day × fish	38	56.01	1.56	0.03
Error	152	35.69		

Although the treatment did not affect the number emerging (between-subjects analysis), juveniles emerged at different rates in ponds with versus those without mosquitofish (within subjects, day × fish interaction; also see Fig. 1). The sequential Bonferroni-corrected alpha level for this test is 0.05.

lected on day 160 showed that most tadpoles suffered tail damage in ponds with fish. None of 12 tadpoles from two fishless ponds showed evidence of injury, but 33 of 34 were missing parts of their tails in a sample of tadpoles from two ponds with fish.

The dominant macroinvertebrates found in ponds represented the orders Odonata (Anisoptera and Zygoptera), Hemiptera (Notonectidae, Corixidae, Belostomatidae, Velliidae and Gerridae), Diptera (Chironomidae, Culicidae), Coleoptera (Dytiscidae, Hydrophilidae), Ephemeroptera (Baetidae), and Gastropoda. Invertebrate densities were lower in ponds with mosquitofish (repeated-measures ANOVA on the total numbers of invertebrates in samples from ponds with versus without fish: $F = 6.20$, df $= 1,10$, $p < 0.05$). Invertebrate abundances increased more over time in ponds without fish than in ponds with fish (day-by-treatment interaction: $F = 2.99$, df $= 1,10$, $p < 0.008$). Mosquitofish reduced the abundances of predatory invertebrates (Fig. 3 top panel, repeated-measures ANOVA, treatment effect: $F = 75.20$, df $1,10, p < 0.001$; this difference is significant with a Bonferroni-corrected alpha of 0.025). We were unable to detect an effect of mosquitofish on grazing invertebrates (repeated-measures ANOVA, day-by-treatment interaction: $F = 1.37$, df $= 7, 70$, $p = 0.229$; treatment effect: $F = 1.04$, df $= 1, 10, p = 0.331$).

The negative effects of mosquitofish on predatory invertebrates were further confirmed by our observations

Table 3.   Results of two single-factor ANOVAs on the effects of bullfrogs and mosquitofish on the weight of juvenile red-legged frogs at metamorphosis.*

Source	df	Mean square	F	p
Bullfrogs	1	0.04	3.56	0.132
Error	4	0.01		
Mosquitofish	1	0.30	22.03	0.009
Error	4	0.01		

Data were ln-transformed before analysis. The sequential Bonferroni-corrected alpha level for these tests are 0.05 (bullfrogs) and 0.025 (mosquitofish).
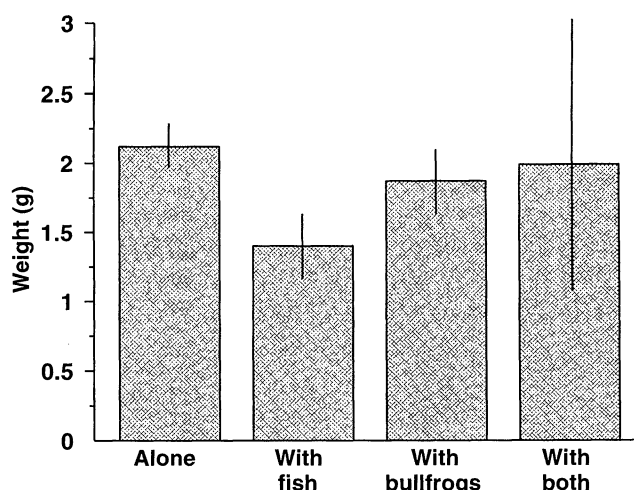
Figure 2. Mean weights of surviving red-legged frog tadpoles raised in ponds alone, with mosquitofish, with bullfrog tadpoles, and with both mosquitofish and bullfrog tadpoles. Error bars are standard deviations.

of notonectids and our collection of odonate exuvia on 17 July. We were not able to see any notonectids in ponds with mosquitofish, whereas we observed at least five notonectids in 25% of the pond surface in all ponds without mosquitofish. Exuvia of the dragonfly *Anax* were more abundant in ponds without mosquitofish (ANOVA: $F = 12.48$, df $= 1, 10, p < 0.005$). *Anax* is a large predator; mean exuvium length in our ponds was 4.5 ± 0.4 cm SD.

Laboratory Experiments

Prey-choice trials indicated that the presence of alternative prey might afford some protection to tadpoles in natural ponds. Mosquitofish consumed mosquitoes and corixids in preference to hatchling red-legged frogs (Fig. 4; *Culex* versus tadpoles, $G_p = 77$, df $= 1, p < 0.001$; corixids versus tadpoles, $G_p = 12$, df $= 1, p < 0.001$). Mosquitofish attacked corixids in preference to tadpoles ($G_p = 19.7$, df $= 1, p < 0.001$) but launched similar numbers of attacks against mosquitoes and tadpoles ($G_p = 0.06$, df $= 1$, not significant). Mosquitofish consumed both *Culex* and *Daphnia* in preference to 3-week-old tadpoles (Fig. 4; replicated G test for *Culex* versus tadpoles, $G_p = 108.1$, df $= 1, p < 0.001$; *Daphnia* versus tadpoles, $G_p = 204$, df $= 1, p < 0.001$). All attacks directed at *Culex* or *Daphnia* resulted in the prey being eaten. In contrast, the larger tadpoles were never consumed and were attacked less frequently than alternative prey (attacks on tadpoles versus *Culex*, $G_p = 24.5$, df $= 1$, $p < 0.001$; *Daphnia* versus tadpoles, $G_p = 24.1$, df $= 1$, $p < 0.001$). Goodness-of-fit tests for heterogeneity
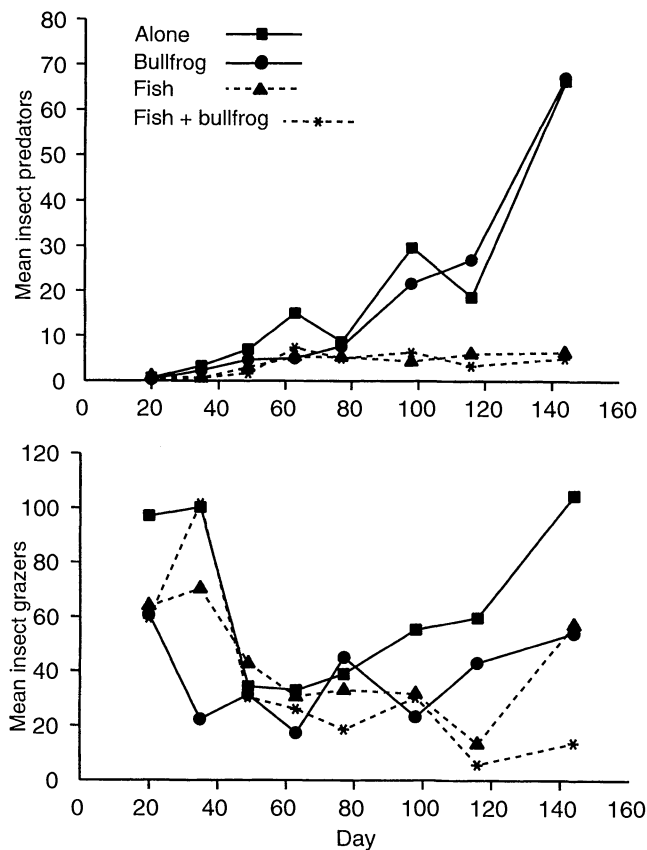
Figure 3. Abundances of invertebrates over time in samples from experimental ponds. Each point represents the average abundance of insects in dipnet samples of three ponds (Alone, red-legged frogs were the only vertebrates; Bullfrog, 50 bullfrog tadpoles were also present; Fish, 8 mosquitofish were also present; and Fish + bullfrog, all three vertebrates were present).

among replicates showed that tadpoles were nonpreferred prey for all but one fish in both experiments. A single fish launched an almost equal number of attacks on *Culex* and tadpoles.

Fish did not kill stage-26 tadpoles when allowed to forage in groups of three during a 16-hour period, but 92% of tadpoles were injured in tanks without alternative prey and 33% of tadpoles were injured in tanks to which *Culex* had been added. A linear model showed that injuries were more frequent in tanks without alternative prey ($p < 0.05$).

The presence of fish caused tadpoles to become less active early in their development, but this effect disappeared at later stages (Fig. 5). Stage-26 tadpoles were 75% less active in the presence of fish than in controls ($\chi^2 = 16.74$, df = 1, $p < 0.001$; treatment explained 54% of the variance). There was no evidence that larger, stage 33–36 tadpoles altered their activity level when fish were present ($\chi^2 = 0.65$, not significant).

Discussion

Our study provides experimental evidence that bullfrogs may play a role in the decline of the California red-legged frog. The presence of just 50 bullfrog tadpoles nearly precluded recruitment of red-legged frog tadpoles to the juvenile stage in our ponds. We did not identify the mechanism underlying this effect, but strong competitive effects of bullfrog tadpoles are often reported for other species. Alternative mechanisms include predation and parasitism or disease. Bullfrog tadpoles will consume tadpoles of other ranids (Ehrlich 1979), but a study by Kiesecker and Blaustein (1997) showed that bullfrog tadpoles consumed northern red-legged frog tadpoles only if the tadpoles converged on another source of food. The study was conducted in aquaria, so it is unclear how often predation occurs in larger habitats. We cannot rule out the possibility that bullfrog tadpoles may have transmitted an infectious agent to the red-legged frog tadpoles, but bullfrog tadpoles appeared healthy when transferred into our ponds, and we did not observe obviously diseased red-legged frog tadpoles in sweep-net samples. Kupferberg (1995) demonstrated experimentally that shared pathogens were unlikely to be the cause of the negative effects of bullfrogs on foothill yellow-legged frogs (*Rana boylii*).

The severe effect of bullfrog tadpoles on red-legged frog recruitment is probably an underestimate of the total effect of introduced bullfrog populations on red-legged frogs because adult bullfrogs eat tadpoles and smaller frogs, including other ranid species (Kupferberg 1995). It is premature to suggest that bullfrogs are the most important agent of red-legged frog decline because the effects of other introduced species have not been quantified, and it is also possible that contaminants or diseases play a role. Nevertheless it was sobering to discover that bullfrog tadpoles alone were capable of nearly eliminating recruitment of the native frog in this experiment. Management strategies aimed at removing adult and juvenile bullfrogs may not be effective if even a few adults escape and breed; egg masses and tadpoles must be removed as well.

Although mosquitofish can eradicate red-legged frog tadpoles from simplified aquatic communities, our experiment showed that mosquitofish do not affect the recruitment of red-legged frogs from more naturalistic, spatially complex, and speciose communities in earthen ponds. Nearly equal numbers of juvenile red-legged frogs emerged from ponds with and without fish, despite dense mosquitofish populations in the former and injuries to the tadpoles that indicated frequent mosquitofish attacks. It is unlikely that tadpoles outgrew predation because mosquitofish can kill any size of red-legged frog tadpole (R. Schmeider & R. Nauman, personal communication). Several factors could have contributed to this result. First, tadpoles are not preferred prey of mosquitofish based on
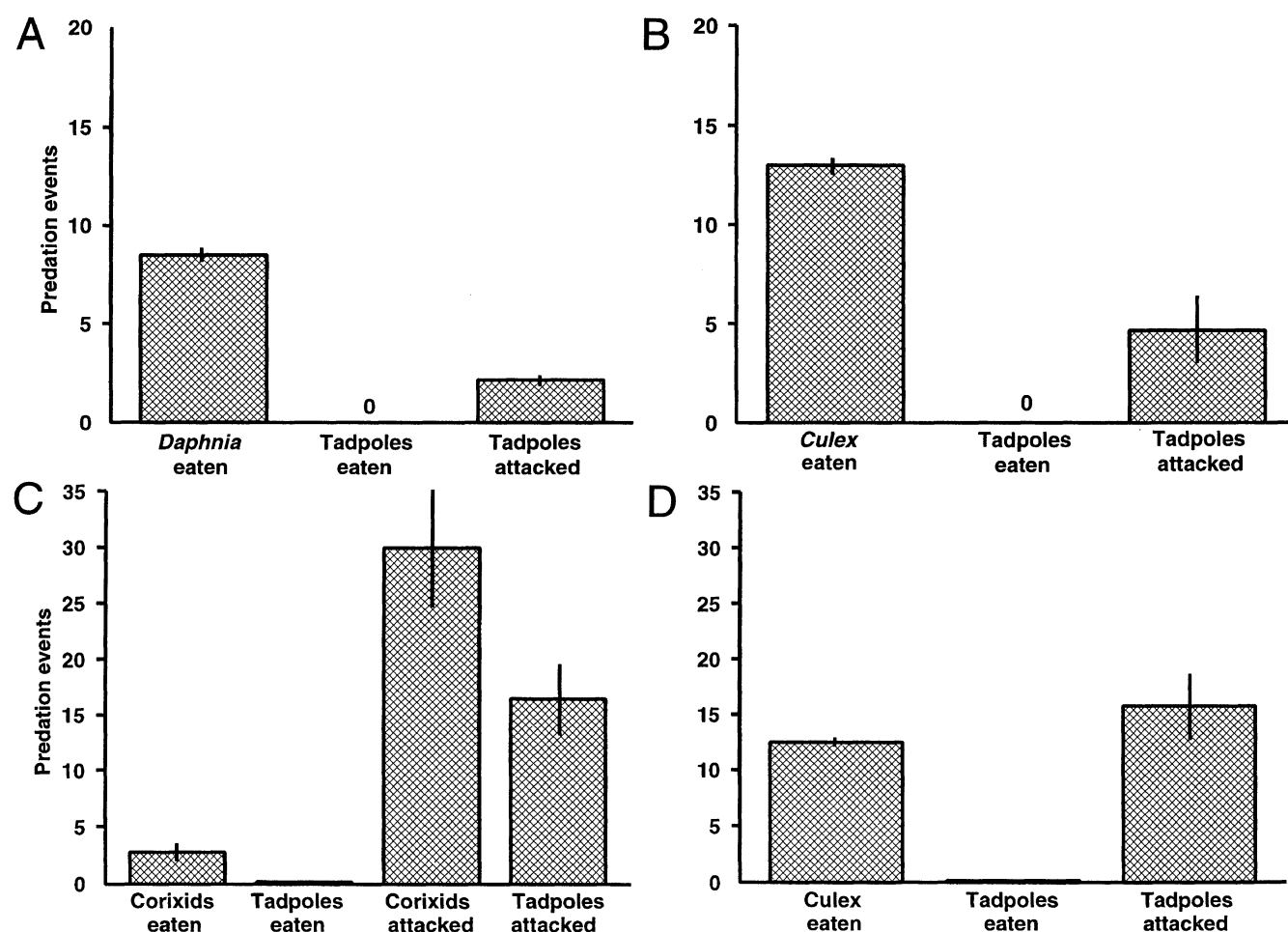
Figure 4. Feeding preference of mosquitofish for red-legged frog tadpoles versus invertebrates. Bars show the average number of predation events (consumption or attacks) per fish by five or six mosquitofish that were allowed to inter-act with prey for up to 30 minutes (A and B, stage-26 tadpoles; C and D, hatchling tadpoles). Error bars are ±1 SE.

laboratory trials, and alternative prey may have protected tadpoles from lethal levels of mosquitofish attacks. Second, ponds contained deep areas, cobbles, and dense vegetation that may have provided refuges for the tadpoles (e.g., Peterson et al. 1992; Babbitt & Jordan 1996). Such spatial heterogeneity is present in many areas where red-legged frogs breed, but our experiment may not predict the effects of mosquitofish in shallow, relatively structure-less pools. Third predaceous invertebrates were less abundant in ponds with fish, and any mortality inflicted by the fish might have been balanced by a decrease in predation from other sources.

Large, predatory dragonflies (*Anax* sp.) were common in ponds without fish. Dragonflies can reduce tadpole populations (Heyer et al. 1975; Smith 1983). Caldwell et al. (1980) found that *Anax* naiads were able to consume tadpoles that were up to 4.9 cm long, which was the maximum size used in their trials. The tadpoles in our study would probably have been vulnerable to dragonfly predation because large *Anax* exuvia were present in
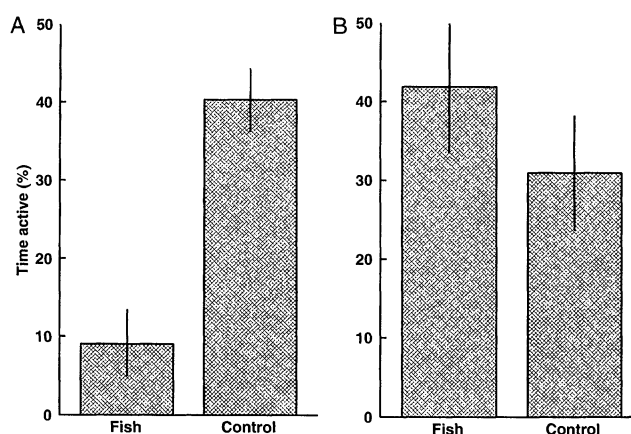

Figure 5. Activity level of red-legged frog tadpoles in the presence and absence of mosquitofish (A, stage-26 tad-poles; B, stage 33–36 tadpoles). Error bars are ±1 SE.

June and continued to appear throughout the summer. Our ponds also held other invertebrate predators, including backswimmers, beetles and beetle larvae, belostomatids (giant water bugs), and damselfly naiads.

We may have underestimated mosquitofish effects if mosquitofish are initially more abundant in natural ponds when tadpoles are small, especially if fish deplete alternative prey prior to frog breeding. Our observations at two riparian sites in Contra Costa and San Joaquin Counties which have both mosquitofish and red-legged frogs suggest that mosquitofish densities in early spring are as low or lower than those in our ponds; these sites are in creeks, however, and some mosquitofish may be flushed out of creeks by winter rains.

Although mosquitofish did not affect amphibian survival in our study, other work by Gamradt and Kats (1996) showed 87% mortality of California newt larvae (*Taricha torosa*) in stream cages where three mosquitofish were caged with four newt larvae for 24 hours ($n = 6$ replicates; cages were $72 \times 45 \times 21$ cm boxes with natural substrate). The abundance of alternative prey was not given, and it is unknown whether cage installation affected alternative prey. These results show that more work is needed to determine the effects of mosquitofish on native fauna.

Although mosquitofish did not affect red-legged frog survival, juveniles emerging from ponds with fish metamorphosed later and weighed an average of one-third less than those raised without fish. Several factors may have contributed to the lower growth rate of tadpoles in ponds with mosquitofish. The laboratory trials showed that young tadpoles were less active in the presence of fish. This could have caused a decrease in their initial growth rate (e.g., Werner 1991; Skelly 1992). Evidence for this mechanism is equivocal because some species of tadpoles also respond to invertebrate predators with decreased activity (e.g., Lawler 1989; Werner 1991), and invertebrate predators were common in ponds without fish. Injuries can also decrease the growth of tadpoles (Wilbur & Semlitsch 1990; Parichy & Kaplan 1992), and injuries were much more common in ponds with fish.

More work is needed to determine whether mosquitofish pose a threat to red-legged frog populations. The smaller metamorphs emerging from ponds with fish might mature later and lay fewer eggs, as has been demonstrated in other amphibians (Smith 1987; Semlitsch et al. 1988). We do not know whether the frogs can grow quickly enough in the terrestrial environment to compensate for their initially smaller size. Because the mosquitofish did have a negative—albeit sublethal—effect on red-legged frogs, it is advisable for mosquito control districts to use other mosquito-control methods in amphibian habitat and surrounding watersheds (e.g., *Bacillus thuringiensis israelensis*, *Lagenidium*, *B. sphaericus*).

Our results illustrate that predation experiments designed to assess the effects of introduced species should include naturalistic levels of spatial and biological complexity. Although we did not do the many detailed experiments necessary to prove that indirect effects or spatial heterogeneity ameliorate the effect of mosquitofish on red-legged frog tadpoles, our results differ markedly from those of predation trials conducted in simplified communities. In addition, our observations and laboratory results demonstrate mechanisms whereby biological complexity could have changed the effect of mosquitofish on red-legged frog tadpoles.

Although simplified tests may measure artifacts, we support their use to screen predators that may be introduced to new areas as biological control agents. Introduced predators can be so damaging that a "precautionary principle" that errs on the side of protecting native species is best, except in rare cases in which the ecological costs of not trying biological control are even greater (Howarth 1991; Simberloff & Stiling 1996; Samways 1997). Laboratory tests often provide a valuable worst-case estimate of effect (although this is not guaranteed because predator behavior may be abnormal in an artificial environment). However, in cases in which several putative causes of a species decline are already in operation, tests of single causes in artificial settings may generate red herrings rather than species recoveries.

Acknowledgments

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