

Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa*

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Abstract

The potential impact of introduced species on rare taxa is of particular concern to conservation biologists. We evaluate the impacts of western mosquitofish (*Gambusia affinis*) and virile crayfish (*Orconectes virilis*) on experimental populations of a threatened species, the White Sands pupfish (*Cyprinodon tularosa*). Forty experimental pupfish populations were exposed to one of four treatments; (a) 1 crayfish, (b) 4 crayfish, (c) 5 adult mosquitofish and (d) control. Pupfish population size and biomass was monitored over the duration of one breeding season. A repeated measure multiple analysis of covariance revealed a significant effect of treatments on response variables (population size and biomass) ($P < 0.0001$). Mosquitofish had a significant effect on population size and biomass ($P = 0.0330$). The effect of one crayfish was not significant ($P = 0.0683$). However, 4 crayfish had a significant effect ($P < 0.0001$) on population size. We use these data, along with information on environmental tolerances of crayfish and mosquitofish, to evaluate risks for specific pupfish populations.

Abbreviations: CF1 – treatment with one crayfish; CF4 – treatment with 4 crayfish; ESU – ecological significant unit; MANCOVA – multiple analysis of covariance; MANOVA – multiple analysis of variance; m – meter; MF – treatment with mosquitofish; PF – control treatment – just pupfish; R – finite growth rate; SD – standard deviation; SE – standard error

Introduction

The impact of non-native species on rare species is one of the greatest threats to global biodiversity (Wilson 1992; Wilcove et al. 1998). This is of particular concern because exotic species are virtually impossible to eliminate once they are established. This places the management burden on preventing the establishment of exotic species. One approach is to assess the risk of establishment and to evaluate potential impacts. The resulting data can allow managers to prioritize efforts on exotic species that pose the greatest threats.

Desert aquatic systems are more at risk from introduced species than other regions in the United States (Sheldon 1988). The potential impacts of exotic species on desert aquatic systems are of special concern because such systems can have relatively high levels of endemism (Soltz and Naiman 1980; Cole 1981; Williams et al. 1985). Indeed, many fish extinctions have been associated with the introduction of exotic species (Miller et al. 1989; Richter et al. 1997; Minckley et al. 2002). One hypothesis for the potential impact of exotics is that desert fishes evolved in a species poor environment with few fish predators or

competitors (Minckley and Douglas 1991). For instance, the Monkey Spring pupfish (*C. arcuatus*) became extinct following the introduction of non-native largemouth bass, *Micropterus salmoides* (Minckley et al. 1991, 2002).

Two exotic species of particular concern are the mosquitofish (*Gambusia* sp.) and crayfish (*Orconectes virilis*), both of which have been widely introduced throughout the western United States (Fuller et al. 1999). Earlier workers have shown that the mosquitofish may impact certain desert fish species (Schoenherr 1981; Meffe 1985; Galat and Robertson 1992). Introduced crayfish species in general negatively impact native species (Gamradt and Kats 1996; Lodge et al. 2000). However, most assessments of exotic species impacts are anecdotal (Courtenay and Meffe 1989) and may often be confounded by other anthropogenic impacts (Moyle and Williams 1990). Here, we take an experimental approach to evaluate potential impacts of virile crayfish (*O. virilis*) and western mosquitofish (*Gambusia affinis*) on the White Sands pupfish (*Cyprinodon tularosa*), a New Mexico threatened species. The White Sands pupfish is endemic to the Tularosa Basin of south central New Mexico, USA, where it occurs in three systems on the White Sands Missile Range and in one habitat on Holloman Air Force Base. Non-native species have been identified as an important threat to White Sands pupfish by the White Sands Pupfish Conservation Team (Stockwell 2002). However, the potential impact of such species on pupfish populations is unknown.

Western mosquitofish and virile crayfish have been introduced to the Tularosa Basin and have sufficient salinity tolerance to invade two of the four pupfish habitats; Malpais Spring and Mound Spring. Populations of mosquitofish and crayfish currently occur within 10 km of Malpais Spring and within 30 km of Mound Spring. Here, we report the impact of virile crayfish and mosquitofish on experimental populations of White Sands pupfish.

Study system

The White Sands pupfish inhabit four separate systems; Malpais Spring, Salt Creek, Lost River and Mound Spring. Only the two former popula-

tions are native, with the Lost River and Mound Spring populations derived by translocation of fish from Salt Creek (Stockwell et al. 1998; Pittenger and Springer 1999). It has been recommended that the Salt Creek and Malpais Spring populations be managed as separate evolutionary significant units (Stockwell et al. 1998). Salt Creek and Lost River have areas of salinity approaching three times that of saltwater which reduces the likelihood of invasion by virile crayfish or mosquitofish (Ahuja 1964; Al-Daham and Bhatti 1977). Malpais Springs is a relatively low salinity spring (and associated marsh system) that is within the environmental tolerances of mosquitofish and crayfish. Another concern is that the Malpais evolutionary significant unit (ESU) has not been replicated, whereas the Salt Creek ESU has been replicated at two sites (Stockwell et al. 1998). Thus, it is imperative to evaluate the potential impacts of exotics species on the Malpais ESU.

This study was initiated to determine if mosquitofish and crayfish pose a credible threat to the White Sands pupfish. Mesocosms were used to examine the effects of mosquitofish and crayfish invasions on pupfish population dynamics. Pupfish from Malpais Springs were used as this spring system is at the greatest risk from exotic introductions.

Materials and methods

In May of 2002, pupfish were collected at the Malpais Spring complex, and mosquitofish were collected from Camera Pad Pond. Crayfish were collected at Guilez Springs. All organisms were weighed and measured prior to use in the experiment. Fish size is often positively correlated with reproductive output (Moyle and Cech 2000). To reduce the possibility of fish size confounding the results of this experiment, only pupfish between 25 and 38 mm were used in the mesocosms. Fish and crayfish were transported to North Dakota State University where a mesocosm experiment was conducted from June 1 to October 7, 2002.

Mesocosms were constructed of plastic wading pools 1.56 m in diameter and approximately 25 cm in depth. These types of mesocosms have been successfully used for raising pupfish (Stock-

well, pers. comm.), and mosquitofish (Hurlbert et al. 1972; Leberg 1990; Mulvey et al. 1995; Rogowski 1997). These mesocosms are realistic habitats as pupfish are often found naturally in isolated pools and channels smaller than our experimental mesocosms. Mesocosms contained between 302 and 370 l of water. Instant Ocean Salt[®] was used to maintain salinities approximately equal to that of Malpais Spring, around 3.5. Mesocosms were established two weeks prior to the introduction of pupfish to allow for a variety of invertebrates and algae to become established.

Each mesocosm was stocked with enough gravel (2–5 mm in size) to cover the bottom (approximately 30 l by volume), a liter of organic material (sediment and detritus) from a local pond, half a liter of pond water, and 1.52 linear meters of artificial cover (plastic breeding substrate). Water from a local water body was used to seed all experimental mesocosms. Water levels were maintained by rainfall and periodic additions of de-chlorinated tap water. Supplemental feeding occurred three times a week, using approximately 0.6 g of Tetramin[®] brand flake food for each feeding.

Pupfish and treatments were randomly assigned to mesocosms over a four day period, 10 mesocosms were stocked per day. Exotic organisms were introduced two weeks after the pupfish were introduced. There were four treatments as presented in Table 1, with the “pupfish only” mesocosms as the control.

Measurements

Water temperature was measured with temperature loggers (Onset Computer Corp. 1996) at 4-h intervals in 8 randomly selected mesocosms for the duration of the experiment. In addition, temperature, conductivity and salinity were measured once a week in every mesocosm.

Population sampling was conducted at 54, 83, and 128 days after pupfish establishment, corresponding to July 22, August 20, and October 4, 2002. Each sample period lasted 4 days, with 10 mesocosms sampled per day. Mesocosms were sampled in the order that they were established. To collect fish, each mesocosm was seined to depletion by dragging a 1.18 mm mesh seine through the pool seven times. All adult fish were measured and weighed separately. Pupfish and mosquitofish that were too small to measure individually (fry and juveniles) were weighed en-masse.

Mesocosms were the unit of replication for all data analyses. However after the second sampling period, fish and crayfish from one mesocosm were inadvertently returned to the wrong mesocosm. As a consequence, subsequent data from these two mesocosms were not included in population analyses. As our sample sizes were not equal, all multivariate and univariate analyses used type three sums of squares (Sokal and Rohlf 1995).

Crayfish densities were not static. Mesocosms with four crayfish declined in density throughout the experiment. By the end of the experiment six mesocosms had three crayfish, three mesocosms had three crayfish, and one mesocosm had one crayfish left. By contrast, only one mesocosm of low density crayfish suffered mortality, and this occurred sometime between August 20 and before the final sample was collected.

Crayfish mortality appeared to be associated with low temperatures that occurred near the end of the experiment. We observed that crayfish movement was severely restricted in temperatures below 8 °C. Crayfish had no means of escaping (burrowing) low temperatures in our mesocosms. By the end of the experiment the actual density of crayfish in the high density treatments was less than the initial density of 2 crayfish per square meter.

Table 1. Experimental design of the White Sands pupfish and introduced species experiment.

Treatment	Pupfish	Introduced organisms	Replicates
Pupfish only (control)	16	0	10
Mosquitofish + pupfish	16	5 mosquitofish (3 females, 2 males)	10
Low density crayfish + pupfish	16	1 crayfish	10
High density crayfish + pupfish	16	4 crayfish	10

Experimental conditions

Salinity varied from 1.3 to 7.6 throughout the experiment with a mean of 3.3 (SD 0.75). There were no significant differences in average salinity of the mesocosms among treatments ($F_{3,36}=0.415$, $P=0.743$).

Mesocosm temperatures ranged from 1.15 to 36.59 with a mean of 20.79 °C over the duration of the experiment. Temperature profiles of all pools were exceptionally similar. The average mean temperature difference of the mesocosms (± 0.26 °C) approximated the accuracy of the data loggers (± 0.2 °C) (Onset Computer Corp. 1996).

The experimental mesocosms provided a fair representation of White Sands pupfish native habitat. This was evidenced by the fact that fish reproduced successfully in the mesocosms. A variety of invertebrates and algae were present in all mesocosms. Although not quantified the macro-invertebrate biota appeared to be similar among mesocosms. Common groups of aquatic macro-invertebrates present included: Libellulidae (Odonata), Naucoridae, Hydrophilidae, Chironomidae, Dytiscidae, Hydrachnida, Corixidae, Notonectidae, Ephydriidae.

Statistics

Differences in population growth rates, biomass, population size, sex ratios, and fish condition were assessed. All statistical analyses were conducted using SAS 8.02 or JMP 5.01a (SAS Institute Inc. 1999).

A repeated measures multivariate analysis of covariance (MANCOVA) was used to determine if there were treatment differences. There was a significant difference in initial pupfish biomass between treatments ($F_{3, 36}=5.021$, $P=0.005$) and accordingly a significant difference in the mean weight and length of pupfish used to stock treatments (results not reported). To control for possible effects of pupfish size on reproduction, initial pupfish biomass was used as a covariate in the analysis.

Population data were log transformed to better approximate a normal distribution. Pupfish population size and biomass for each sampling period (1, 2, and 3) were used as dependent

variables, with initial biomass as a covariate. Time period zero for population size was not included in the model as there was no variation in the founding number of pupfish. Finite growth rates (R) of populations were calculated and log transformed to better approximate a normal distribution for use in an analysis of variance. Planned contrasts included control mesocosms against each of the treatments.

Condition (α) was calculated for individual fish using the allometric growth rate model ($\alpha = ((\text{standard length})^\beta / \text{wet weight}) * 10$) (Desiro 1999). Unit length is in centimeters, and weight is in grams. Beta (amount of curvature) was estimated from a non-linear regression by sex and sampling period for control pupfish using an iterative non-linear fitting process (SAS Institute Inc. 1999). Treatments were compared to controls using a beta that was derived from the control pupfish for that particular sampling period and sex. Pupfish with a standard length of 27–37 mm were used to investigate fish condition. This size restriction reduced potential problems associated with condition indices when a wide range of lengths are used (Bolger and Connolly 1989). A repeated measures multiple analysis of variance (MANVOVA) was used to investigate differences in mean condition of pupfish by mesocosm. For condition analyses, four time periods were used; when fish were first introduced (initial condition of founding fish), and the three subsequent sampling periods.

Adult population size was investigated through a MANOVA. Pupfish that were 25 mm or more in standard length were classified as adults. This was the minimum size of the initial founding fish.

Results

All pupfish populations survived the duration of the experiment. Treatment had a significant effect on the response of population size and biomass (Table 2). The covariate, initial biomass did not have a significant effect on the response measures. Response of high density crayfish treatments, and mosquitofish treatments, had significantly lower population size (Figure 1) and biomass (Figure 2) than the controls (Table 2).

Treatment had a significant effect on pupfish population growth rates (Table 3). Population growth rates in the high crayfish density and mosquitofish treatments were significantly lower than in the control (Figure 3), but there was no difference between the low density crayfish and the control (Table 3, Figure 3). There was no significant relationship between the number of mosquitofish and pupfish ($F_{1,7} = 1.2201$, $P = 0.3058$).

Condition

Pupfish condition factor was invariant with standard length, for each sampling period and sex ($P > 0.35$, for all cases). There was a significant difference between the condition of male and female pupfish (Table 4). Male pupfish had a greater condition factor than female pupfish (Figure 4). There was a significant interaction between sex and time. Female condition increased throughout the experiment whereas male condition was nearly static the last two sampling periods. There was no significant effect of treatment on pupfish condition.

Population size of adults

There was a significant difference in the number of adults over time (Table 5), and by treatment (Table 6). The numbers of adults in high density crayfish treatments were significantly less than in

the controls. The other treatments were not significantly different from each other.

Discussion

The results of this study provide good experimental evidence that two widely introduced species, *G. affinis*, and *O. virilis*, are likely to have detrimental effects on wild populations of White Sands pupfish. Population growth rate and biomass of pupfish was significantly reduced in the presence of mosquitofish, and crayfish at high density.

Mosquitofish had a detrimental effect on pupfish populations. It is not known whether this was a result of mosquitofish predation (on pupfish eggs or fry), competition for limited resources, or a combination of factors. Mosquitofish did quite well in these mesocosms. Average population growth rate of mosquitofish in the presence of pupfish was 14.12 (SE 1.96) compared to pupfish population growth rate of 1.001 (SE 0.062) in the mosquitofish treatments. In one mesocosm by the end of the experiment there were over 7 times as many mosquitofish as there were pupfish. The average ratio of mosquitofish to pupfish was 4.39, with a biomass ratio of 1.40. However, there was not a significant relationship between the number of mosquitofish and number of pupfish.

Table 2. Summary MANOVA results, test criteria and exact F statistics for the hypothesis of no response (pupfish population size and biomass) by treatment, time, interactions, and planned contrasts.

	Wilks' Lambda	F -value	Num. DF	Den. DF	P
Response*treatment	0.2719	9.79	6	64	< 0.0001
Response* initial biomass	0.9506	0.83	2	32	0.4446
Planned contrasts of response*treatments, (control vs. exotics)					
Control vs one crayfish	0.8456	2.92	2	32	0.0683
Control vs four crayfish	0.3183	34.27	2	32	< 0.0001
Control vs mosquitofish	0.8080	3.80	2	32	0.0330
Response*time	0.8718	1.10	4	30	0.3732
Response*time*treatment	0.4324	2.47	12	79.664	0.0084
Time*initial biomass	0.8722	1.10	4	30	0.3750
Planned contrasts of response*time*treatment, (control vs exotics)					
Control vs one crayfish	0.7202	2.91	4	30	0.0378
Control vs four crayfish	0.6928	3.33	4	30	0.0228
Control vs mosquitofish	0.6294	4.42	4	30	0.0063

Num. = numerator; Den. = denominator; DF = degree of freedom; P = probability.

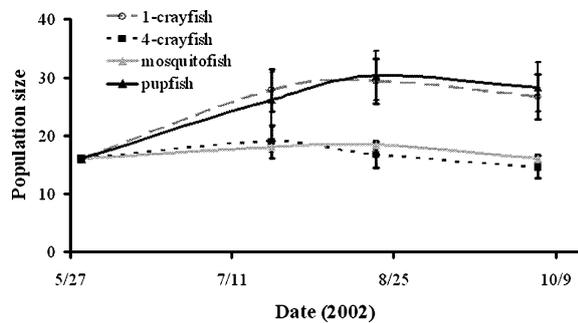


Figure 1. Population size of White Sands pupfish by treatment with standard error bars.

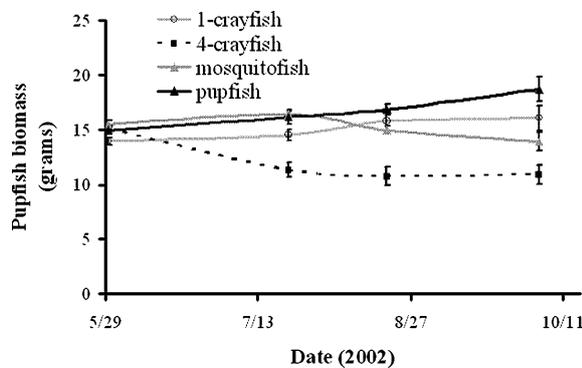


Figure 2. Biomass of White Sands pupfish by treatment with standard error bars.

Pupfish populations exposed to crayfish at densities of about $2/m^2$ (our high density treatment) would be unlikely to maintain viable populations. Reproduction and survival of pupfish populations exposed to 4 crayfish were reduced. Biomass of pupfish in the high crayfish density treatments was significantly less than the other treatments. On several occasions crayfish were seen consuming adult pupfish in the high density crayfish treatments.

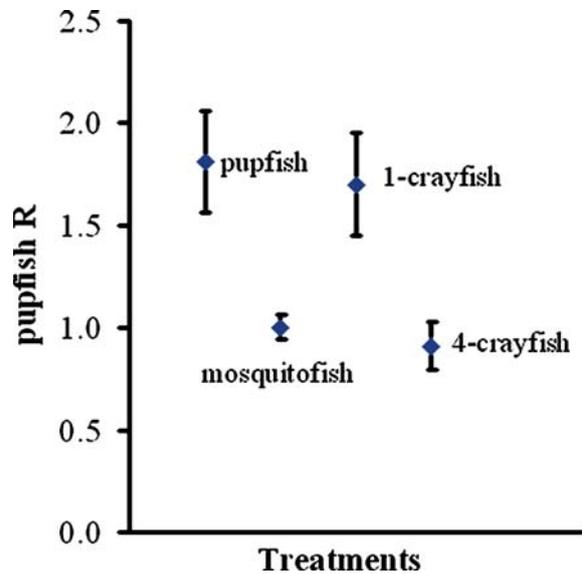


Figure 3. Mean population growth rate of White Sand pupfish over a summer breeding season (4 months) and standard error bars.

Mechanisms for reduced population growth rate were not explicitly tested in this experiment; however, pupfish condition data and the number of adult pupfish provide insights into potential mechanisms of reduced population growth. A lack of a treatment effect on condition suggests that resource availability did not differ among the treatments.

Adult population size remained fairly constant in all treatments with the exception of the high density crayfish treatment. There was some mortality and recruitment within the adult population in a number of mesocosms. In some mesocosms, large founding females were no longer observed in subsequent population censuses, although adult population size remained constant. In addi-

Table 3. ANOVA of pupfish population growth rate (log transformed) by treatment and planned contrasts.

Source	DF	Sum-of-squares	Mean square	F-ratio	P
Treatment	3	0.594	0.198	7.464	0.001
Error	34	0.903	0.027		
Contrast	DF	Contrast sum-of-squares	Mean square	F-value	P
Control vs 1 crayfish	1	0.005891	0.005891	0.22	0.6407
Control vs 4 crayfish	1	0.4260	0.4260	16.04	0.0003
Control vs mosquitofish	1	0.2466	0.2466	9.289	0.0045

$N = 38$ multiple, $R = 0.630$, squared multiple $R = 0.397$; DF = degree of freedom, $P =$ probability

Table 4. Summary MANOVA test criteria and exact F statistics for the hypothesis of no difference in pupfish condition, by sex, treatment, time and their interactions.

	Wilks' Lambda	F -value	Num. DF	Den. DF	P
Response (sex)	0.0006843	24096.7	2	33	< 0.0001
Response (sex)*treatment	0.80379	1.27	6	66	0.2836
Response (sex)*time	0.0040937	1175.84	6	29	< 0.0001
Response (sex)*time*treatment	0.47546	1.38	18	82.51	0.1649

Num. = numerator, Den. = denominator, DF = degree of freedom, P = probability.

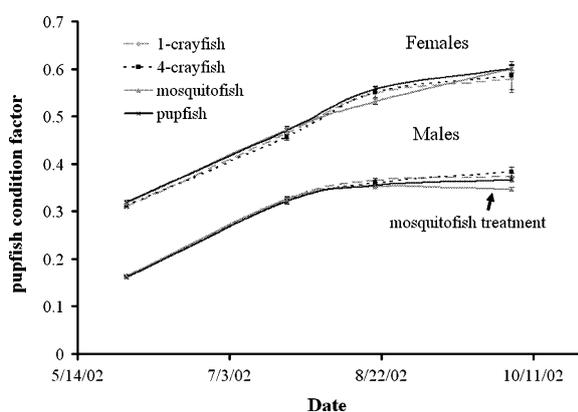


Figure 4. Condition factor of adult White Sands pupfish by sex and treatment over one breeding season with standard error bars. Only fish between 26 and 38 mm were used in this comparison.

tion, 5 mesocosms had an initial decline in adults, followed by an increase later in the experiment.

Results of the condition and adult population size analyses suggest that the primary cause of reduced population growth rate in pupfish is recruitment failure. Mosquitofish predation of eggs or juvenile fish is suspected as the primary reason behind reduced population growth rate. Mosquitofish predation on eggs/juvenile fish is in concordance with findings from previous researchers (Schoenherr 1981; Meffe 1985; Lydeard and Belk 1993).

Table 5. Summary MANOVA test criteria and exact F statistics for the hypothesis of no difference in number of adult pupfish by time, treatment, and their interactions.

	Wilks' Lambda	F -value	Num. DF	Den. DF	P
Time	0.52104	15.17	2	33	< 0.0001
Time*treatment	0.72060	1.96	6	66	0.0844

Num. = numerator, Den. = denominator, DF = degree of freedom, P = probability.

Our data suggest that the introduction of mosquitofish would have a detrimental impact on wild populations of White Sands pupfish. These potential impacts are especially critical for the unreplicated Malpais Spring ESU. Thus, management should aim to reduce the risk of introduction of mosquitofish. This would most effectively be accomplished by the eradication of the limited number of current mosquitofish populations in the Tularosa Basin (estimated at fewer than 10).

The potential impacts of crayfish by contrast appear to be density dependent. If crayfish density could be kept lower than 0.5/m, pupfish might be able to maintain viable populations. Crayfish densities in the wild (Massachusetts pond and marl lakes in northern Michigan) have been estimated at 1.9–6.1 crayfish per square meter (Camougis and Hichar 1959; Momot et al. 1977). The high reproductive potential of *O. virilis* (up to 443 eggs per female) (Momot et al. 1977) suggests that preventing establishment may be better than control.

As long as mosquitofish remain in the same basin as pupfish there is a potential for them to be introduced. Indeed, non-regulated introduction of fish has been very common throughout the United States (Welcomme 1992; Fuller et al. 1999; Benson 2000). Further, along with the mosquitofish and crayfish, two of the current populations of pupfish (Pittenger and Springer 1999) are the result of non-regulated translocations. We have also observed introduced large-mouth bass (*M. salmoides*) and sunfish (unknown sp.) in springs within the Tularosa Basin.

We only investigated a small aspect of the effects of introduced mosquitofish and virile crayfish on one species within the Tularosa Basin. Aquatic organisms in this area have been little studied and the potential effects of these exotics on other species are unknown. Introduced cray-

Table 6. Summary of repeated measures analysis of between subject effects and planned contrasts of the number of adult pupfish.

Source	DF	Sum-of-squares	Mean square	F-ratio	P
Treatment	3	468.833	165.278	30.36	0.001
Error	34	175.026	5.148		
Contrast	DF	Contrast sum-of-squares	Mean square	F-value	Pr > F
Control vs 1 crayfish	1	12.576	12.576	2.44	0.1273
Control vs 4 crayfish	1	350.417	350.417	68.07	< 0.0001
Control vs mosquitofish	1	0.00312	0.00312	0.00	0.9805

DF = degree of freedom, P = probability.

fish have been known to alter habitat to the detriment of native species (Lodge et al. 2000; Stenroth and Nystrom 2003).

This has been an experimental test of the apparent threat that mosquitofish and crayfish pose to the White Sands pupfish. Many desert aquatic systems are fairly small and can be adequately replicated in mesocosms. Mesocosms allow for experimental tests of the potential effects of introduced species as advocated by Vermeij (1996). Many endemic fishes and invertebrates from desert aquatic systems can be successfully used in mesocosms experiments. For species with short life spans, population growth rate can be a moderately simple metric, and a direct measure of fitness. If populations decline in the presence of an introduced species, than the exotic species can be considered detrimental and efforts should be made to ensure that it does not become established. Experimental designs such as used in this study can help guide and prioritize conservation efforts towards the most cost effective measures.

Our research has confirmed our suspicion that mosquitofish and crayfish can be detrimental to pupfish populations. Conservation efforts should be directed at preventing these exotics from establishing in pupfish habitats. Once invasive species are established they can be impossible or prohibitively expensive to remove (Simberloff 1997; Pimentel et al. 2000; Simberloff 2003).

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