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NOTE

An Experimental Test of Novel Ecological Communities of Imperiled and Invasive Species

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Abstract

Imperiled fish species are often managed by establishing refuge populations as a hedge against extinction, but suitable sites are often at a premium. Thus, managers may wish to consider novel strategies, such as establishing multispecies refuges that already include undesirable species. To determine the suitability of multispecies refuges, we established experimental communities that included allopatric and sympatric communities of three fish species: the endangered Pahrump Poolfish *Empetrichthys latos*, the Amargosa Pupfish *Cyprinodon nevadensis*, and the invasive Western Mosquitofish *Gambusia affinis*. Mosquitofish juvenile production was not significantly affected by the presence of the other species (mean ± SE: 50 ± 18 in allopatry, 33 ± 6 with poolfish, and 38 ± 7 with both poolfish and pupfish). Similarly, pupfish persisted in sympatry with both poolfish and mosquitofish, but pupfish had higher juvenile production when maintained in allopatry (557 ± 248) and in the presence of poolfish (425 ± 36) than in the presence of both poolfish and mosquitofish (242 ± 32). By contrast, poolfish juvenile production was high in allopatry (123 ± 17) but significantly lower in the presence of pupfish (6.6 ± 1.2) and mosquitofish (1.0 ± 0.5) individually and in a community of all three species (0.5 ± 0.4). This suggests that translocated pupfish can coexist in refuges containing nonnative mosquitofish but that endangered poolfish are not compatible with the other species and the current management of poolfish in single-species refuges is appropriate. Consequently, our results indicate that multispecies refuges are suitable for some endangered species, which will give managers more latitude in the management of these species.

The conservation of biodiversity often requires active management, such as the establishment of ex situ refuges (i.e., refuges maintained for conservation purposes outside of the taxon's native environment) as a hedge against extinction (Griffith et al. 1989; Minckley 1995; Wolf et al. 1996; Olden 2011). While conservation refuges have become an important tool for the management of many species in North America's southwestern deserts (Pister 1993; Minckley 1995; Ostermann et al. 2001; Deacon and Williams 2011), such actions are often constrained by the lack of suitable habitats, especially in arid regions where aquatic habitats are at a premium (Moyle and Sato 1991; Minckley 1995). For protected southwestern fishes, single-species refuges are typically established in fishless springs or artificial habitats (Dunham and Minckley 1998; Karam et al. 2012). Single-species refuges have been preferred because many protected southwestern fishes evolved in simple communities with few or no other fish species being present (Miller 1948; Soltz and Naiman 1978) and thus may be naïve to potential predators and/or competitors (Meffe 1985; Cox and Lima 2006). Thus, sites harboring invasive nonnative species are typically considered unsuitable as refuge habitats for protected fish species (Henkanaththegedara and Stockwell 2014).

In general, nonnative species are detrimental to the persistence of imperiled desert fish (e.g., Meffe 1985; Marsh and Langhorst 1988). However, recent work has shown that the impacts of invasive species may not be universally negative and that the degree of compatibility may be condition specific, involving abiotic as well as biotic factors (Dunson and Travis 1991; Henkanaththegedara and Stockwell 2012, 2014). For instance, intraguild predation (predation on potential competitors) may allow co-persistence among native and nonnative species (Lenon et al. 2002; Henkanaththegedara and Stockwell 2012, 2014),

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thereby increasing management opportunities for protecting rare species. For instance, one intriguing option would be to establish multispecies refuges at sites that already harbor other native species or even nonnative species (Mueller 2006). However, information will be required to evaluate the likelihood of co-persistence for the targeted species.

In this paper, we consider the prospect of multispecies refuges by focusing on species from the Cyprinodontidae and Goodeidae families that have been actively managed by using refuges: Amargosa Pupfish *Cyprinodon nevadensis* and Pahrump Poolfish *Empetrichthys latos*. Both of these species evolved in isolated perennial springs in the Death Valley hydrological system (Miller 1948), often in habitats and fish communities that are not as complex as others in North America. Many pupfishes have been managed in ex situ refuges (e.g., Miller and Pister 1971; Baugh and Deacon 1988; Hendrickson and Romero 1989; Dunham and Minckley 1998). Similarly, the Pahrump Poolfish has been managed in single-species refuge sites since 1971 (Deacon and Williams 2011). Establishing additional refuge populations would assist the recovery of this species. However, potential refuge habitats often are inhabited by species such as Western Mosquitofish *Gambusia affinis*, a nonnative invasive that is listed as a threat to Amargosa Pupfish (USFWS 1990), and habitats with mosquitofish have been considered unsuitable as poolfish refuge habitats (USFWS 1980).

While appealing, multispecies refuges for desert fish have rarely been tested (however, see Robinson and Ward 2011). A first step in testing the multispecies concept is to understand compatibility among key species. In this paper we examine compatibility based on species-specific juvenile production within experimental communities of Pahrump Poolfish, Amargosa Pupfish, and Western Mosquitofish raised in sympatry Q2 and allopatry. We performed this experiment in a seminatural mesocosm using species and habitats that can be considered proxies for similar species and potential ex situ refuges.

METHODS

Fish were wild caught from Spring Mountain Ranch State Park, Clark County, Nevada (Pahrump Poolfish); Crystal Spring, Nye County, Nevada, and Little Alkali Spring, Mono County, California (Western Mosquitofish); and River Springs, Mono County, California (Amargosa Pupfish). Q3 Allopatric and sympatric communities of poolfish, mosquitofish, and pupfish were maintained in mesocosms at an outdoor field site on the North Dakota State University campus in Cass County.

Experimental fish communities were assigned to circular 1,211-L rigid plastic tubs. Gravel substrate and artificial cover material (five 0.5-m-long clumps of plastic mesh weighted to simulate rooted aquatic plants) were added to all tubs to create structure. These mesocosms were inoculated with a mixture of plankton from a local, semipermanent wetland, covered with wire mesh, aerated, and maintained at a water volume of approximately 700 L. The treatments included experimental fish communities that consisted of one, two, or all three species.

We focused our efforts on understanding the effects of Western Mosquitofish and Amargosa Pupfish on Pahrump Poolfish due to immediate conservation needs and the fact that this combination of native species has been proposed by managers for future refuges. We established 10 replicates of the following four experimental communities: (1) allopatric poolfish, (2) poolfish and pupfish, (3) poolfish and mosquitofish, and (4) poolfish, pupfish, and mosquitofish. To obtain additional insights on the reciprocal effects of poolfish on the other two species, we established three replicates of the following communities: (5) allopatric mosquitofish and (6) allopatric pupfish. Nine adults of each species were introduced into each experimental community. We randomly selected six females and three males for both the pupfish and mosquitofish. The initial sex ratio for poolfish was unknown because it is difficult to definitively determine the sex in this species. However, because poolfish are sexually dimorphic by size (unpublished data), we haphazardly selected a mixture of sizes to ensure that there was a mixture of sexes in each mesocosm. To limit competition and comply with IACUC requirements, every day Q4 fish were fed a mixture of aquarium flake and crushed koi pellets in a quantity equivalent to 5% of their stocked mass.

Water conditions and quality were monitored. Water temperatures changed relative to environmental conditions following a diel rhythm as well as over the course of the experiment. The experiment was terminated at 71 d, at which time all fish were removed from the tubs and euthanized with 500 mg/L of tricaine methanesulfonate (MS-222; Western Chemical, Inc.). Fish were then preserved in 10% formalin, identified, sexed, and counted. We recorded the number of surviving juveniles per species and treatment as a measure of productivity. We also recorded the number of juveniles per surviving adult female for the Pahrump Poolfish and Amargosa Pupfish but did not do so for the Western Mosquitofish because the final number of adult female mosquitofish could have included both parental and first-generation adult females.

Treatment comparisons were analyzed (SPSS; IBM Corp.) using the Kruskal–Wallis *H*-test, and the experiment-wise error rate was maintained at 0.05 using sequential Bonferroni Q5 (Rice 1989).

RESULTS

The mesocosms appeared to provide adequate environmental conditions for survival and reproduction. In many cases the number of adult Western Mosquitofish exceeded the number of founders due to the recruitment of first-generation offspring. Thus, estimating the adult survival of mosquitofish was not possible. Average adult survival across the allopatric and sympatric treatments varied from 85% to 89% for Pahrump Poolfish and from 76% to 100% for Amargosa Pupfish (Table 1).

TABLE 1. Average survival (%) of adult Amargosa Pupfish and adult Pahrump Poolfish per treatment in experiments involving Western mosquitofish; n.a. = not available.

Treatment	Poolfish survival	Pupfish survival
Allopatric poolfish	89	n.a.
Allopatric pupfish	n.a.	100
Poolfish and pupfish	88	94
Poolfish and mosquitofish[a]	86	n.a.
Poolfish, pupfish, and mosquitofish[a]	85	76[b]

[a]The adult survival of mosquitofish was not estimated because the final number of adults included both founding and first-generation fish.

[b]If the tank with only one pupfish survivor is excluded, average pupfish survival was 83%.

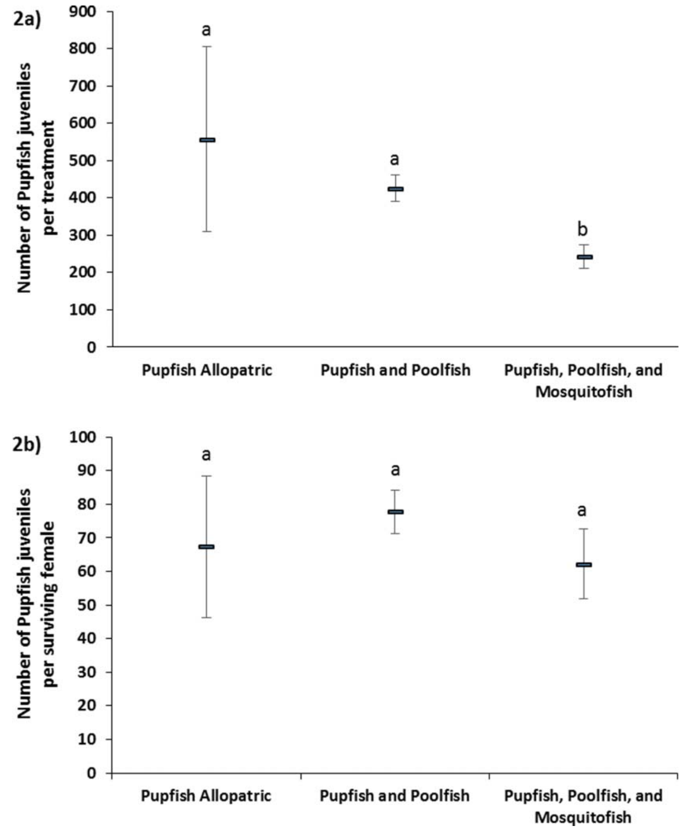

FIGURE 2. Juvenile production of Amargosa Pupfish **(a)** per treatment and **(b)** per surviving female. See Figure 1 for additional information.

The lower survival for pupfish was associated with one mesocosm with all three species in which there was only one surviving adult pupfish. We excluded this tank from the additional analyses of juvenile production of all three species. The final sex ratio for poolfish varied from 11% to 75% female, but the number of surviving poolfish females did not differ significantly among treatments (H = 1.825, df = 3, P = 0.609).

All three species successfully reproduced when in allopatry. The number of Western Mosquitofish juveniles per mesocosm did not differ significantly among treatments (H = 0.578, df = 2, P = 0.749). There were 253 ± 95 (mean ± SE) mosquitofish juveniles in allopatry, compared with 180 ± 31 when mosquitofish were sympatric with only Pahrump Poolfish and 187 ± 27 when they were sympatric with both poolfish and Amargosa Pupfish (Figure 1).

The number of Amargosa Pupfish juveniles in allopatry (557 ± 248) did not differ from that when they were sympatric with Pahrump Poolfish (425 ± 36), but both values were significantly higher than the number of pupfish in the presence of both poolfish and Western Mosquitofish (242 ± 32) (H = 8.87, df = 2, P = 0.012; Figure 2a). However, juvenile production per female did not differ among the three treatments (H = 1.032, df = 2, P = 0.597; Figure 2b).

Pahrump Poolfish sympatric with Amargosa Pupfish and/or Western Mosquitofish were severely limited in terms of recruitment. The number of poolfish juveniles was significantly higher in allopatry (123 ± 17) than when poolfish were sympatric with pupfish (7 ± 1), mosquitofish (1 ± 0.5), or both species (0.5 ± 0.4) (H = 26.591, df = 3, P = 0.000), but there were no significant differences in poolfish juvenile production among the three sympatric communities (Figure 3a). The number of poolfish juveniles per female was significantly higher in allopatry (55 ± 16) than when poolfish were sympatric with pupfish (3 ± 0.7), mosquitofish (0.6 ± 0.3), or both pupfish and mosquitofish (0.25 ± 0.2) (H = 25.104, df = 3, P < 0.001; Figure 3b). There were no differences in the number of poolfish juveniles per female among the three sympatric communities.

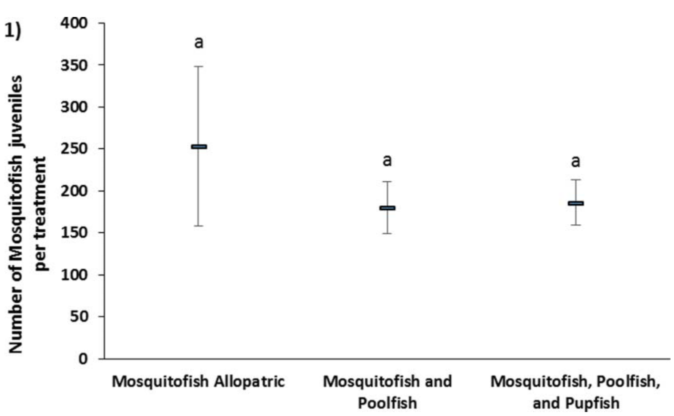

FIGURE 1. Juvenile production per treatment for Western Mosquitofish. The dark horizontal bars represent the means, the error bars the SEs. Means with the same lowercase letter are not significantly different from each other.

DISCUSSION

Novel multispecies refuges are an appealing solution to the challenge of protecting species when refuge habitats are at a

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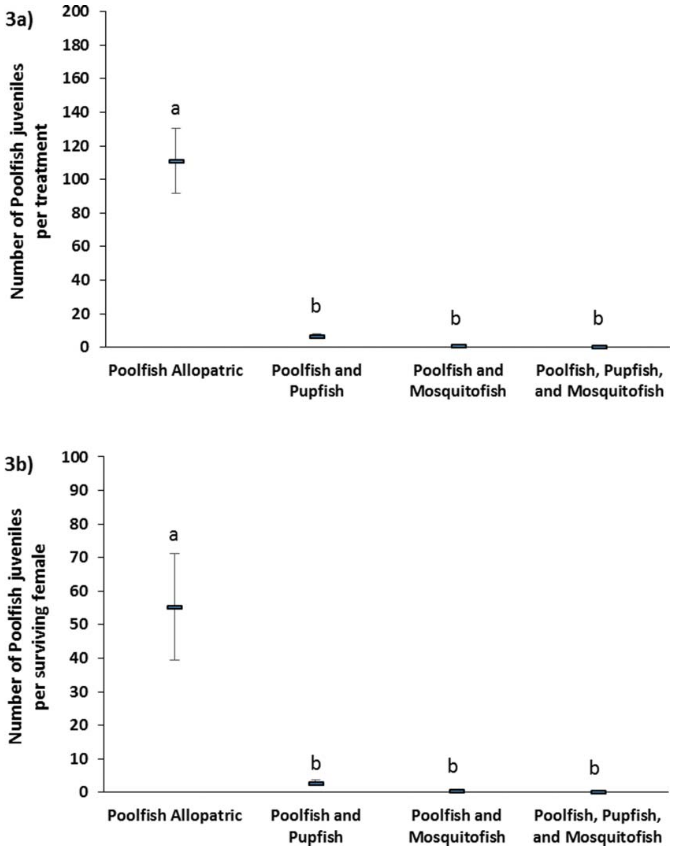

FIGURE 3. Juvenile production of Pahrump Poolfish **(a)** per treatment and **(b)** per surviving female. See Figure 1 for additional information.

premium. Such an approach will require that all species of concern can co-persist; however, the findings from this experiment suggest that there are differences among species in their potential to co-persist with other fish species.

According to our results, Amargosa Pupfish may be able to co-persist with other species such as Pahrump Poolfish and Western Mosquitofish. In fact, the number of pupfish produced per female was not affected by the presence of the other two species. These findings are contrary to those of earlier work by Rogowski and Stockwell (2006), who reported that mosquitofish negatively affected the population growth of experimental populations of the White Sands Pupfish *C. tularosa*. However, the current study involved a different pupfish species and larger experimental habitats than those used by Rogowski and Stockwell (2006).

In contrast to Amargosa Pupfish, Pahrump Poolfish were not able to get established in the presence of pupfish and/or Western Mosquitofish. The study habitats were sufficient to support high poolfish production when the species was allopatric; however, poolfish juvenile survival was virtually zero in the presence of pupfish and/or mosquitofish. The lower survival was presumably due to predation on poolfish eggs and/or larvae—rather than competition with the other species—because food was provided.

Our findings represent a first step in understanding how these species may interact in a multispecies refuge, but we recognize some limitations of our experimental design. First, we used an "additive" experimental design in which total abundance increases in tandem with the addition of more species. Fausch (1998) suggested that additive designs are best suited to situations in which species differ in ecology or size (Fausch 1998). Western Mosquitofish are surface-feeding livebearers (Pyke 2004), whereas Amargosa Pupfish and Pahrump Poolfish are pelagic and benthic feeders, which did not allow us to disentangle the effects of intraspecific competition from those of interspecific competition. Further, because we examined juvenile production as the response variable, a substitutive design (in which the overall density of fish is equal among treatments) would impose other limitations because the number of adults producing eggs/larvae would vary among treatments. Q7 Q8

Second, our findings only show that Amargosa Pupfish and Western Mosquitofish populations may co-persist in the short term. Thus, more work will be necessary to assess the long-term potential for co-persistence. The short duration of our experiment is grossly comparable to the peak breeding seasons of all three species in southern Nevada, making our study length biologically relevant. It is noteworthy that populations of the Ash Meadows subspecies of Amargosa Pupfish *C. n. mionectes* have co-persisted with invasive Western Mosquitofish for many decades (La Rivers 1994). Importantly, our findings show that Pahrump Poolfish do not co-persist with pupfish or mosquitofish even in the short term.

Our findings for the Pahrump Poolfish experiment are consistent with the stated concern that exotic fishes are a threat to poolfish recovery (USFWS 1980) and suggest that novel multispecies refuges may not be a viable option for conserving this species. However, additional research should be conducted before ruling out multispecies refuges as an option for conserving Pahrump Poolfish. For instance, it is possible that poolfish will be able to co-persist with Amargosa Pupfish and/or Western Mosquitofish in more spatially complex habitats. It is important to note that the closely related Ash Meadows Poolfish *E. merriami* historically co-occurred with the Ash Meadows subspecies of Amargosa Pupfish and the Ash Meadows Speckled Dace *Rhinichthys osculus* ssp. in complex habitats (La Rivers 1994). Q9

Our findings, however, suggest that Amargosa Pupfish may be able to co-persist with Western Mosquitofish. If so, the labor-intensive removal of mosquitofish may not be necessary to establish and maintain pupfish refuge populations. Finally, our results suggest that novel multispecies refuges could expand conservation options, and this possibility deserves further exploration.

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