EX SITU ANALYSIS OF NON-NATIVE SPECIES IMPACTS ON IMPERILED DESERT FISHES

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ABSTRACT

This thesis focuses on interactions between two invasive species, the western mosquitofish (*Gambusia affinis*) and the red swamp crayfish (*Procambarus clarkii*), and two desert fishes, the Amargosa pupfish (*Cyprinodon nevadensis*) and the endangered Pahrump poolfish (*Empetrichthys latos latos*).

Chapter one is a literature review on the impacts of invasive species on various desert fishes. Chapter Two reports the results of multiple mesocosm experiments that show poolfish are unlikely to persist in the presence of mosquitofish. Chapter Three reports the results of a mesocosm experiment that tested the combined impacts of crayfish and mosquitofish on poolfish populations. Chapter Three mimics a historic case study in Nevada, where the Lake Harriet poolfish population crashed following the introduction of crayfish and mosquitofish. Chapter Four reports results of a mesocosm experiment that revealed density dependent effects of invasive crayfish on pupfish populations, with pupfish populations negatively affected when crayfish were at high densities.

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DEDICATION

I dedicate this work to friends and family that believed in me, specifically my close friends that I have had across the years and all the amazing people I met during my time at NDSU.

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CHAPTER 1. LITERATURE REVIEW ON PAHRUMP POOLFISH

During the Pleistocene Era, the southwest region of the United States was comprised of many rivers, natural springs, and vernal pools (Soltz and Naiman, 1978). During this period, the formation of major headwaters and rivers lead to the recharge of major pluvial lakes and other water bodies (Enzel, 2003). As the Pleistocene ended, pluvial lakes desiccated due to reduced recharge rates and increased temperatures (Smith, 1940, Soltz and Naiman, 1978). The receding water levels resulted in a series of fragmented aquatic ecosystems containing isolated pools and springs that varied in salinity, temperature, and flow. The newly formed aquatic habitats contained native species isolated in numerous diverse ecosystems (Brown, 1971; Soltz and Naiman, 1978). The shift in environmental characteristics within the region set the stage for rapid evolutionary diversification (Miller 1948, 1961; Collyer et al. 2011; Stockwell et al. 2013).

One classic example of rapid diversification can be seen within the pupfishes of the Death Valley region. This region hosts the ‘Western’ Cyprinodon clade which diverged from other Cyprinodon in the early Pleistocene ~1.9 Mya (Echelle et al., 2008). Subsequently, rapid divergence occurred among populations that were isolated among diverse habitats at the end of the Pleistocene. This system hosts four pupfish species (Cyprinodontidae: Cyprinodon) including eight distinct subspecies, which shared ancestry sometime during the Pleistocene (Miller 1948, 1981; Echelle et al. 2005; Echelle and Echelle, 1993).

The Death Valley system also hosts a lesser known killifish genus *Empetrichthys*, that contained two species; *E. merriami,* and *E. latos*, including 3 subspecies within *E. latos* (Miller 1950). These taxa were restricted to small warm springs all hosted within a limited geographical range in western Nevada. The Ash Meadows poolfish (*E. merriami*) historically occurred within Ash Meadows, Nevada where it was sympatric with the Ash Meadows Amargosa pupfish (*C.*

nevadensis mionectes) and the Amargosa speckled dace (*Rhynichthys osculus nevadensis*)(La Rivers 1962). The other group of poolfish species distinct from *E. merriami* contained three subspecies (*E. latos* ssp), all of which resided in the neighboring Pahrump Valley, Nevada (Miller, 1950). The close geographical proximity of subspecies within desert fishes occurs due to the hypothesized connections of the Amargosa River, connecting Death Valley and Pahrump valley before water levels had receded (Echelle, 2005, 2008). Causes for species differentiation were likely driven by genetic drift and/or adaptive divergence (Miller, 1950; Sheffer et al., 1997; Echelle et al., 2005, 2008). The rapid diversification of pupfishes among divergent habitats has led some ichthyologists to refer to pupfishes as the Galapagos fishes of the southwest (Soltz and Naiman 1978).

The endemic species of Southwestern North America are solely found within this region. This makes the desert fishes a concern for conservation biology. Rapid anthropogenic changes have resulted in many extinctions of endemic fish taxa and numerous other species. Population decline leads to increased extinction risk (Pister, 1974; Miller et al., 1989). In the book, "*Battle Against Extinction*" the authors state that there are two places in America where extinction rates are more than twice the rate of catastrophic geological extinctions, one being western America (Udall, 2017). These extinctions were directly associated with alterations to the landscape, such as water diversion for agriculture, increased water consumption by growing populations, nutrient pollution, and invasive species introductions (Smith, 1940; Soltz and Naiman 1978; Sada, 1990; Cucherousset and Olden, 2017).

The loss of water resources within this region has had immediate and important impacts on many aquatic species. The subfamily Empetrichthydae was particularly impacted through water diversion, and irrigation for agricultural usage. In 1958, the Pahrump Ranch poolfish (*E. l.*

pahrump) was driven to extinction when its sole spring was pumped dry to accommodate increased irrigation rates (Minckley and Deacon, 1968). A close relative, the Raycraft Ranch poolfish (*E. l. concavus*), also nearly lost its habitat due to ranchers filling the springhead, an attempt to control mosquitoes in the area (Minckley and Deacon, 1968). Eventually, the increased number of water wells drilled in the region led to extinction of *E. l. concavus* in 1959 (Minckley and Deacon, 1968; Miller et al., 1989). The last poolfish subspecies in this valley persisted in its native habitat at Manse Spring, until it went dry in 1975. Fortunately, this species persists today because Dr. James Deacon had the foresight to transplant poolfish to two refuge habitats in 1970 and 1971 (Jimenez et al., 2017).

We see another example of how water alterations in this region impacted native fishes within the Gila topminnow (*Poeciliopsis occidentalis*). This species was once believed to be the most common species in the southern Colorado Basin. However, it has been reduced to 9 total locations within the entire United States (Minckley and Deacon, 1968; Sheffer et al., 1997). In addition to habitat degradation, desert fishes such as the Gila topminnow were also severely impacted by the introduction of invasive fishes (Miller 1961; Meffe, 1985; Sheffer et al., 1997). The Gila topminnow (*Poeciliopsis occidentalis*) was locally extirpated when invasive Western mosquitofish were introduced (Meffe, 1985). Another invasive species, the largemouth bass (*Micropterus salmoides*), played a key role in driving extinction of the Monkey Springs pupfish (*Cyprinodon sp.*). These cases along with the decline of numerous local fish populations demonstrate the vulnerability of desert fishes to invasions (Miller et al. 1989). Desert fish are believed to be vulnerable due to their naivety to predation, as numerous desert fishes have evolved in single species habitats (Miller, 1948; Meffe 1985).

Many of the invasive fish species became established in the southwest during the late 1930's (Deacon et al., 1964; Miller, 1961; Minckley and Deacon 1968; Stockwell et al 1996). Two invasive species that had significant impact on many native species are the western mosquitofish (*Gambusia affinis*) and its close relative the eastern mosquitofish (*Gambusia holbrooki*), collectively referred to as mosquitofish. These species were introduced into the southwestern United States as biological control agents for mosquitoes during the early 1900's, reaching California in 1922 and subsequently Nevada in the 1930's (La Rivers, 1994; Stockwell et al., 1996).

Mosquitofish have broad environmental tolerances, capable of surviving within ecosystems with variable salinity and temperature (Meffe and Snelson, 1989; Laha, 2006; Catat, 2013). Mosquitofish are opportunistic feeders, consuming algae, invertebrates, juvenile and larval fishes, and are occasionally cannibalistic (Speczir, 2004). These species are also known to have large brood sizes of up to 100 larvae per clutch, with juveniles reaching maturity in 3-4 weeks (Krumholz, 1948; Pyke, 2008).

Rapid population growth combined with opportunistic feeding behaviors may explain the broad and significant impacts of mosquitofish on the ecosystems they invade (Minckley and Deacon, 1968; Moyle, 1976; Schoenherr, 1981). For example, Mills et al. (2004) examined mosquitofish impacts on the least chub (*Iotichthys phlegethontis*) and found that high densities of mosquitofish resulted in no survival for least chub young of the year. Environmental factors such as temperature may mediate impacts by invasive species such as mosquitofish. For instance, water bodies with spatial heterogeneity in temperature facilitate the coexistence of least chub with invasive western mosquitofish, as the chub can utilize cooler water (<15ºC), while mosquitofish will avoid these areas (Ayala et al., 2007; Priddis et al., 2009). Priddis et al. (2009)

found that overwinter survival was significantly lower for mosquitofish (~45% adult survival) compared to least chubs (~75% adult survival). Nevertheless, rapid maturity combined with rapid reproduction allowed mosquitofish to severely impact the least chub's juvenile productivity.

For endemic species such as the least chub, the impacts of invasive competitors and predators have been attributed to predator naïveté. The naïveté of many desert fish species is attributed to evolution within simple ecological communities containing few fish predators or competitors, and therefore presumably native fish have lost anti-predator traits (Miller, 1948; Meffe 1985). Such impacts have been shown by numerous experimental studies, demonstrating that invasive mosquitofish negatively impact juvenile productivity of desert fishes (Meffe, 1985; Mills et al., 2004; Rogowski and Stockwell 2006; Goodchild and Stockwell 2016). For example, mosquitofish have directly impacted the productivity of Gila topminnow through consumption of topminnow offspring (Meffe, 1985). Many other desert fishes have seen population declines, and/or eventual extirpation due to predation following the invasion of mosquitofish and other invasive species such as various species of crayfish (Miller, 1961; Deacon et al., 1964; Shoenherr, 1981; Williams, 1985; Stockwell and Henkanaththegedara 2011).

In addition to invasive fish, various species of invasive crayfish may also threaten the recovery of desert fishes (Williams, 1985; Guan, 1997, 1998; Rogowski and Stockwell 2006; Thomas and Taylor, 2013). Crayfish can rapidly colonize a region. A single pregnant female can lay up to 400 eggs biannually, resulting in rapid population growth within invaded systems (Oluoch, 1990). Crayfish are known to be omnivorous and will prey upon fish opportunistically. Thomas and Taylor (2013) demonstrated that the rusty crayfish (*Orconectes rusticus*) diet included benthic fish (~12%). Such impacts may have played a role in the decline of numerous endemic fishes. For instance, extinction of the Ash Meadows killifish was concurrent with the introduction of invasive red swamp crayfish (*Procambarus clarkia*; Miller, 1989).

Some desert aquatic systems have been invaded by both numerous fish species and crayfish. In fact, both red swamp crayfish and western mosquitofish appear to have impacted the endangered Pahrump poolfish (*Empetrichthys latos latos*). This species is the last living member of the sub-family Empetrichthyinae, and a close congener to the extinct Ash Meadows killifish. The Pahrump poolfish currently only resides in refuge populations as it was extirpated from its only native habitat, Manse Spring (Deacon, 1964). The extirpation of poolfish from Manse Spring resulted from habitat modifications and the introduction of invasive goldfish (*Carassius auratus*) (Deacon and Williams, 2010). Goldfish introductions led to interspecific competition with poolfish for resources. Biologists were unsuccessful in eradicating goldfish, as it was thought that only a few goldfish survived and were capable of recolonizing Manse Spring. (Deacon and Williams, 2010). Ultimately, the combined effects of goldfish and uncertain water management led managers to relocate the remaining individuals to refuges in Las Vegas Valley (Jimenez et al., 2017).

Since the early 1970's, Pahrump poolfish have been managed among a few refuge habitats in Nevada which have experienced periodic extirpations followed by assisted re-colonization (Goodchild 2016; Jimenez et al. 2017). For instance, the Corn Creek refuge population was extirpated in the early 1990's due to the illegal introduction of both crayfish and bullfrogs (USFWS, 2004).

Mosquitofish are one of the invasive species that have been considered a threat to the recovery of Pahrump poolfish (USFWS, 1980). Thus, prior to reintroduction of poolfish into natural habitats, mosquitofish extirpation has been recommended. These recommendations were based only on the documented impacts of mosquitofish involving another fish species. Goodchild and Stockwell (2016) reported that, while mosquitofish did not impact adult poolfish survival, they did have dramatic impacts on poolfish juvenile productivity presumably through consumption of both eggs and juvenile poolfish (Goodchild and Stockwell 2016).

The precarious status of the Pahrump poolfish led to research considering creative management options, such as co-managing protected species such as poolfish and pupfish in multi-species refuges to optimize use of limited aquatic habitats in the southwest deserts (Goodchild and Stockwell 2016). This management option is of interest because historical and pre-historic fish communities were most likely were more complex, however still unlikely had large piscivorous species. In fact, the Ash Meadows Amargosa pupfish (*C. n. mionectes*) historically co-occurred with the Ash Meadows killifish (*E. merriami*), a congener of the Pahrump poolfish. To test the viability of this management option, Goodchild and Stockwell (2016) tested if poolfish could co-persist with another fish species. They found that poolfish had trivial juvenile productivity rates when sympatric with either mosquitofish or pupfish.

The low survival of poolfish larvae in the presence of pupfish was unexpected, as closely related congeners have co-persisted historically. The outcomes may have been due to the simplified spatial complexity of the experimental mesocosms used by Goodchild and Stockwell (2016). This led me to ask if alternative contexts may allow poolfish to co-persist with mosquitofish. I hypothesized that increased habitat complexity would benefit poolfish juvenile productivity by creating refuge sites for juveniles. We decided to increase spatial habitat as Deacon and Miller (2011) attributed the population crash at Manse Spring to removal of aquatic vegetation (Deacon et al., 1964; Deacon and Williams, 2011). The following chapters are manuscripts coauthored by myself and other members of the Stockwell Research Laboratory. In chapter two, we empirically test the interactions between mosquitofish and poolfish among three distinct contexts including; a vulnerability model to predation, variation amongst two phenotypically distinct mosquitofish populations, and relative increased spatial complexity. This is done to assess the repeatability of effects among different contexts, as reproducibility amongst numerous contexts will provide management insight for best action following invasions (Arts et al., 2015; Baker, 2016; Bavel et al., 2016). Specifically, this chapter reports the results of two experiments. In one experiment, two distinct phenotypic variants of mosquitofish were utilized to determine if phenotype resulted in variation within effects on experimental poolfish populations (Goodchild 2015). In a second experiment, we re-evaluated mosquitofish impacts on poolfish populations within mesocosms containing increased spatial habitat complexity. This experiment was pursued because the original work reported by Goodchild and Stockwell (2016) had demonstrated that poolfish were not capable of co-persisting with either invasive western mosquitofish nor endemic Amargosa pupfish. This idea was pursued as increased habitat complexity has been shown to increase juvenile biomass and survival within marine ecosystems (Dunson and Travis, 1991; Graham and Nash, 2012).

Invasion of regions by multiple species has become common; however, little is known about the combined effects of invasive species within desert ecosystems. Anecdotal evidence suggests that both western mosquitofish and red swamp crayfish may impact Pahrump poolfish within refuge habitats. In addition to the Corn Creek population crash in 1998, the Lake Harriet refuge habitat was invaded by crayfish in 2012 and mosquitofish in 2016. Prior to the establishment of crayfish and mosquitofish, the Lake Harriet population had upwards of 10,000 Pahrump poolfish individuals but declined to 161 individuals following the introduction of crayfish and mosquitofish (Kevin Guadalupe, 2008 NDOW Report). Subsequently, the remaining poolfish were transplanted to the Lake Mead Fish Hatchery.

The potential impacts of crayfish on Pahrump poolfish have not been experimentally tested. Previous studies focused on benthic fishes that utilize similar water depths as crayfish. These studies have shown that benthic fishes are vulnerable to crayfish predation (Rogowski and Stockwell, 2006; Thomas and Taylor, 2013). However, Pahrump poolfish are a pelagic species, therefore they are expected to have lower encounter rate with benthic crayfish. In Chapter Three, we tested the effects of crayfish on experimental poolfish populations. We also tested the combined effects of crayfish and mosquitofish on poolfish in order to mimic the dual invasion of red swamp crayfish and western mosquitofish at Lake Harriet.

To compliment this work, we evaluated the density-specific impacts of crayfish on experimental populations of the Amargosa pupfish. This work was motivated by a conflict in the literature that showed different effects of crayfish on benthic fishes. Rogowski and Stockwell (2006) showed that crayfish in high densities had the largest impact on the adult survival and juvenile production of experimental populations of the White Sands pupfish (*C. tularosa*). In a more recent study, Thomas and Taylor (2013) showed that conspecific conflict among crayfish at high densities reduced their impacts on native fishes. At high densities, crayfish were shown to have higher encounter rates with conspecifics leading to higher intra-species conflict which in turn reduced predation rates upon benthic fishes (Thomas and Taylor, 2013). Differences in observed density specific interactions in these two studies may reflect the usage of different species of crayfish (Virile crayfish *Orconectes virilis* and Signal crayfish *Pacifastacus leniusculus* respectively) or reflect experimental conditions.

To further evaluate the effects of density and interference competition on pupfish, we utilized a mesocosm design, where density of crayfish was directly manipulated. To directly measure effects of crayfish density, both low- and high-density treatments were utilized following similar methodology as used by Rogowski and Stockwell (2006). We also tested if interference competition may explain the inverse relationship between crayfish density and fish survival reported by Thomas and Taylor (2013). To test this hypothesis, we included variation of the high-density crayfish treatment, by using tethered crayfish and untethered crayfish, we assumed that tethering crayfish would limit conspecific interactions.

1.1. References

Advocates, W. R. 2006. Water in the urban southwest: An updated analysis of water use in Albuquerque, Las Vegas valley, and Tucson. Boulder (CO): Western Resource Advocates.

Ayala, J. R., R. B. Rader, M. C. Belk, and G. B. Schaalje. 2007. Ground-truthing the impact of invasive species: Spatio-temporal overlap between native least chub and introduced western mosquitofish. Biological Invasions 9:857-869.

Benson, A. J., and Fuller, P. L. 1999. Nonindigenous crustaceans in the United States. Proceedings of the Zebra Mussel and Other Aquatic Nuisance Species Conference Duluth, MN.

Brown, J. H. 1971. The desert pupfish. Scientific American 225:104-113.

Carmona-Catot, G., K. Magellan, and E. Garcia-Berthou. 2013. Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. PLoS One 8:e54734.

Deacon, J. E., C. Hubbs, and B. J. Zahuranec. 1964. Some effects of introduced fishes on the native fish fauna of southern Nevada. Copeia :384-388.

Deacon, J. E., A. E. Williams, C. D. Williams, and J. E. Williams. 2007. Fueling population growth in Las Vegas: How large-scale groundwater withdrawal could burn regional biodiversity. AIBS Bulletin 57:688-698.

Deacon, J. E., and J. E. Williams. 2011. Retrospective evaluation of the effects of human disturbance and goldfish introduction on endangered Pahrump poolfish. Western North American Naturalist 70:425-436.

Dix, K. 1977. Work relations in the coal industry: The hand loading era, 1880-1930.

Echelle, A. A., E. W. Carson, A. F. Echelle, Van Den Bussche, Ronald A, T. E. Dowling, and A. Meyer. 2005. Historical biogeography of the new-world pupfish genus Cyprinodon (Teleostei: Cyprinodontidae). Copeia 2005:320-339.

Echelle, A. A., and A. F. Echelle. 1993. Allozyme perspective on mitochondrial DNA variation and evolution of the Death Valley pupfishes (Cyprinodontidae: Cyprinodon). Copeia :275-287.

Enzel, Y., S. G. Wells, and N. Lancaster. 2003. Late Pleistocene lakes along the Mojave river, southeast California. Special Papers-Geological Society of America:61-78.

Evans, J. P., A. Pilastro, and I. Schlupp. 2011. Ecology and evolution of poeciliid fishes. University of Chicago Press.

Foster, S. A., V. B. Garcia, and M. Y. Town. 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the three-spined stickleback. Oecologia 74:577-585.

Fryxell, D. C., A. R. Diluzio, M. A. Friedman, N. A. Menge, and E. P. Palkovacs. 2016. Cross-habitat effects shape the ecosystem consequences of co-invasion by a pelagic and a benthic consumer. Oecologia 182:519-528.

Gergel, S. E., M. G. Turner, J. R. Miller, J. M. Melack, and E. H. Stanley. 2002. Landscape indicators of human impacts to riverine systems. Aquatic Sciences 64:118-128.

Goodchild, S. C. (2015). Life History and Interspecific Co-Persistence of Native Imperiled Fishes in Single Species and Multi-Species Ex Situ Refuges. Ph.D. dissertation, North Dakota State University, Fargo, ND.

Guan, R., and P. R. Wiles. 1997. Ecological impact of introduced crayfish on benthic fishes in a British lowland river. Conservation Biology 11:641-647.

Guan, R., and P. R. Wiles. 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. Aquaculture 169:177-193.

Gumm, J. M., J. L. Snekser, and M. Itzkowitz. 2008. Conservation and conflict between endangered desert fishes. Biology Letters 4:655-658.

Gumm, J. M., J. L. Snekser, J. M. Leese, K. P. Little, J. K. Leiser, V. E. Imhoff, B. Westrick, and M. Itzkowitz. 2011. Management of interactions between endangered species using habitat restoration. Biological Conservation 144:2171-2176.

Henkanaththegedara, S. M., and C. A. Stockwell. 2013. The role of gape-limitation in intraguild predation between endangered Mohave tui chub and non-native western mosquitofish. Ecology of Freshwater Fish 22:11-20.

Henkanaththegedara, S. M., and C. A. Stockwell. 2014. Intraguild predation may facilitate coexistence of native and non-native fish. Journal of Applied Ecology 51:1057-1065.

Hubbs, C. L., and R. R. Miller. 1948. The zoological evidence: Correlation between fish distribution and hydrographic history in the desert basins of western united states. University of Utah.

Hunt, C. B. 1966. Hydrologic basin, Death Valley, California. US Government Printing Office.

Jimenez, M., S. C. Goodchild, C. A. Stockwell, and S. C. Lema. 2017. Characterization and phylogenetic analysis of complete mitochondrial genomes for two desert Cyprinodontoid fishes, Empetrichthys latos and Crenichthys baileyi. Gene 626:163-172.

Karam, A. P., M. S. Parker, and L. T. Lyons. 2012. Ecological comparison between three artificial refuges and the natural habitat for Devils hole pupfish. North American Journal of Fisheries Management 32:224-238.

Kobetich, G. 1980. Pahrump killifish recovery plan. US Fish and Wildlife Service.

Kodric-Brown, A. 1981. Variable breeding systems in pupfishes (genus *Cyprinodon*): Adaptations to changing environments. Fishes in North American Deserts. John Wiley, NY:205-236.

Krumholz, L. A. 1948. Reproduction in the western mosquitofish, *Gambusia affinis* (Baird and Girard), and its use in mosquito control. Ecological Monographs 18:1-43.

La Rivers, I. 1994. Fishes and fisheries of Nevada. University of Nevada Press.

Laha, M., and H. T. Mattingly. 2006. Identifying environmental conditions to promote species coexistence: An example with the native Barrens topminnow and invasive western mosquitofish. Biological Invasions 8:719-725.

Louarn, H., and A. Cloarec. 1997. Insect predation on pike fry. Journal of Fish Biology 50:366-370.

Magoulick, D. D. 2014. Impacts of drought and crayfish invasion on stream ecosystem structure and function. River Research and Applications 30:1309-1317.

Meffe, G. K. 1985. Predation and species replacement in American southwestern fishes: A case study. The Southwestern Naturalist :173-187.

Miller, R. R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada.

Miller, R. R. 1950. Speciation in fishes of the genera Cyprinodon and Empetrichthys, inhabiting the Death Valley region. Evolution 4:155-163.

Miller, R. R. 1961. Man, and the changing fish fauna of the American southwest. Michigan Academy of Science, Arts, and Letters.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14:22-38.

Mills, M. D., R. B. Rader, and M. C. Belk. 2004. Complex interactions between native and invasive fish: The simultaneous effects of multiple negative interactions. Oecologia 141:713-721.

Minckley, W. L., and J. E. Deacon. 1968. Southwestern fishes and the enigma of" endangered species". Science 159:1424-1432.

Minckley, W. L., G. K. Meffe, and D. L. Soltz. 1991. Conservation and Management of Short-lived Fishes: The Cyprinodontoids. Battle Against Extinction: Native Fish Management in the American West 1991:247-282.

Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America 98(10):5446-5451.

Moyle, P. B. 1976. Fish introductions in California: History and impact on native fishes. Biological Conservation 9:101-118

Oluoch, A. O. 1990. Breeding biology of the Louisiana red swamp crayfish *Procambarus clarkii* Girard in Lake Naivasha, Kenya. Hydrobiologia 208:85-92.

Persson, L. 1988. Asymmetries in competitive and predatory interactions in fish populations. Pages 203-218 Size-structured populations. Springer, Berlin, Heidelberg, 1988. 203-218

Pister, E. P. 1974. Desert fishes and their habitats. Transactions of the American Fisheries Society 103:531-540.

Priddis, E., R. Rader, M. Belk, B. Schaalje, and S. Merkley. 2009. Can separation along the temperature niche axis promote coexistence between native and invasive species? Diversity and Distributions 15:682-691.

Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced gambusia species. Annual Review of Ecology, Evolution, and Systematics 39:171-191.

Rogowski, D. L., and C. A. Stockwell. 2006. Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa*. Biological Invasions 8:79-87.

Sada, D. W. 1990. Recovery plan for the endangered and threatened species of Ash Meadows, Nevada. US Fish and Wildlife Service.

Schoenherr, A. A. 1981. The role of competition in the replacement of native fishes by introduced species. In R. J. Naiman and D. L. Soltz (eds.), Fishes in North American Deserts, 173 –203. New York: John Wiley and Sons.

Sheffer, R. J., P. W. Hedrick, W. L. Minckley, and A. L. Velasco. 1997. Fitness in the endangered Gila topminnow. Conservation Biology 11:162-171.

Soltz, D. L., and R. J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History of Los Angeles County.

Stockwell, C. A., J. S. Heilveil, and K. Purcell. 2013. Estimating divergence time for two evolutionarily significant units of a protected fish species. Conservation Genetics 14:215-222.

Stockwell, C. A., M. Mulvey, and G. L. Vinyard. 1996. Translocations and the preservation of allelic diversity. Conservation Biology 10:1133-1141.

Stockwell, C. A., Mulvey, M., and Jones, A. G. 1998. Genetic evidence for two evolutionarily significant units of white sands pupfish. Proceedings of the Animal Conservation Forum 1:213-225.

Stockwell, C. A., and S. M. Henkanaththegedara. 2011. Evolutionary conservation biology. Pages 128-141 *in* Evans, J. P., A. Pilastro, and I. Schlupp, eds. Ecology and Evolution of *Poeciliid* Fishes. University of Chicago Press, Chicago.

Speczir, A. 2004. Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of Lake Hvz, Hungary. Hydrobiologia 522:249-260.

Thomas, C. L., and C. A. Taylor. 2013. Scavenger or predator? Examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. Freshwater Science 32.4: 309-1317.

U.S. Fish and Wildlife Service (USFWS). 2004. Withdrawal of proposed rule to reclassify the Pahrump poolfish (*Empetrichthys latos*) from endangered to threatened status. *Federal Register* 69: 17383- 17386.

Veselý, L., M. Buřič, and A. Kouba. 2015. Hardy exotics species in temperate zone: Can "warm water" crayfish invaders establish regardless of low temperatures? Scientific Reports 5.

Vondracek, B., W. A. Wurtsbaugh, and J. J. Cech. 1988. Growth and reproduction of the mosquitofish, gambusia affinis, in relation to temperature and ration level: Consequences for life history. Environmental Biology of Fishes 21:45-57.

Weyl, O., B. R. Ellender, D. J. Woodford, and M. S. Jordaan. 2013. Fish distributions in the Rondegat River, cape floristic region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. African Journal of Aquatic Science 38:201-209.

Williams, J. E., and D. W. Sada. 1985. Status of two endangered fishes, *Cyprinodon nevadensis mionectes* and *Rhinichthys osculus nevadensis*, from two springs in ash meadows, Nevada. The Southwestern Naturalist 30:475-484.

Wilson, K. A., J. J. Magnuson, D. M. Lodge, A. M. Hill, T. K. Kratz, W. L. Perry, and T. V. Willis. 2004. A long-term rusty crayfish (*Orconectes rusticus*) invasion: Dispersal patterns and community change in a north temperate lake. Canadian Journal of Fisheries and Aquatic Sciences 61:2255-2266.

CHAPTER 2. ARE WESTERN MOSQUITOFISH IMPACTS ON PAHRUMP POOLFISH CONSISTENT? TESTING THE REPRODUCIBILITY OF MOSQUITOFISH IMPACTS AMONGST NUMEROUS EXPERIMENTAL CONTEXTS

2.1. Abstract

We evaluated the co-persistence of endangered Pahrump poolfish with invasive western mosquitofish across different contexts. First, we modeled prey vulnerability for poolfish juveniles when exposed to two distinct mosquitofish populations; smaller-bodied Wabuska and larger-bodied Garrett mosquitofish. A complementary mesocosm experiment revealed that poolfish juvenile productivity was greater in allopatry (33.56 ±.53 juveniles / mesocosm) than when poolfish were sympatric with Garrett (2.2 ± .21) or Wabuska populations of mosquitofish (0.89 ± 0.16; Z= 3.54, P=.0004; Z=3.39, P=.0007, respectively). The latter two treatments did not differ with respect to poolfish juvenile productivity (Z = 0.55; P = 0.58).

We repeated the mesocosm experiment reported by Goodchild and Stockwell (2016); but changed experimental design by enhancing habitat complexity relative Goodchild and Stockwell. We found allopatric poolfish produced (77.6 ± 14.8 juveniles ± standard error) significantly more than sympatry with mosquitofish (4.3 ± 0.9; Z =-3.32, P=.0009). This work across these different settings increased our confidence in concluding that poolfish are unlikely to co-persist with mosquitofish.

2.2. Introduction

Invasive species are expected to have higher impacts on native populations that evolved in simple communities (Miller 1961; Minckley and Deacon 1968; Courtenay and Deacon 1983; Cox and Lima 2006); however, the severity of impacts may be context specific (Pen and Potter, 1991; Henkananththegedara and Stockwell 2012; Carmona-Catot et al., 2013). Understanding how context can mediate the impact of invasive species is a topic of concern for the conservation of endemic species that evolved in simple systems, such as the desert fishes of southwestern North America (Miller 1948; Soltz and Naiman 1978; Deacon and Minckley 1991). While some studies have shown negative impacts of invasive species on native fishes that evolved in simple communities (e.g. Meffe 1985; Marsh and Langhorst 1988; Lowe 2000; Rogowski and Stockwell 2006), other studies have suggested, native species can co-persist with invasive species (Pen and Potter, 1991; Henkananththegedara and Stockwell 2014). For example, the Amargosa pupfish has co-persisted with sailfin mollies (*Poecilia latipinna*), western mosquitofish (*Gambusia affinis*), and red swamp crayfish (*Procambarus clarkii*) within Ash Meadows (Scoppettone et al., 2005). By contrast, other species such as the Gila topminnow (*Poeciliopsis occidentalis*) have been extirpated following the introduction of invasive mosquitofish (Minckley and Deacon, 1968; Simons et al., 1989; Sheffer et al., 1997). The inconsistent findings call for evaluating impacts for single species across different contexts to understand the generality of invasive species impacts on endemic species.

Factors such as autecological characteristics of invasive species and/or habitat complexity may mediate impacts of invasive species on native species (Dunson and Travis 1991; Pen and Potter, 1991; Ayala et al. 2007; Henkananththegedara and Stockwell 2014). For example, autecological characteristics such as the relative body sizes of native and invasive species may mediate the impacts of introduced species. A recent study reported that gape-limited predation by endangered Mohave tui chub (*Gila bicolor mohavensis*) adults on mosquitofish adults dampened reciprocal predation by adult mosquitofish on tui chub larvae (Henkanaththegedara and Stockwell, 2013, 2014). These findings suggest that body size may mediate impacts of invasive species, through gape-limited predation. In fact, body size can differ by two-fold among invasive populations of the western mosquitofish (Stearns, 1983; Stockwell and Weeks 1999; Stockwell and Vinyard, 2000; Ouyang et al. 2018). Stockwell and Weeks (1999) reported evolutionary divergence between Garret and Wabuska populations of mosquitofish, specifically these populations had significant body size and gape associated variation. Such phenotypic divergence may result in high variation of mosquitofish impacts on native species (Palkovacs et al. 2009).

Habitat complexity also can influence co-persistence of native with invasive species (Diehl, 1988; Menge and Lubchenco, 1981; Graham and Nash, 2013). For example, habitat complexity may mediate impacts of invasive species on native species by facilitating predator-release through increased refuge availability for native fishes (Diehl, 1988; Menge and Lubchenco, 1981; Graham and Nash, 2013). Also, spatial habitat complexity may allow co-persistence among native and invasive species (Scoppettone et al., 2005). For example, Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*) utilized faster flowing and warmer areas of King's Pool streams, whereas invasive species utilized cooler water, with this limiting interspecific interactions (Scoppettone et al. 2005).

These observations suggest that studies investigating different environmental characteristics may provide better insights as to whether native species can co-persist with invasive species. This paper focuses on the endangered Pahrump poolfish (*Empetrichthys latos latos*) which evolved in allopatry in Manse Spring, Nye County, Nevada. Prior to the failure of

Manse Spring, 29 poolfish were translocated to the Corn Creek refuge habitat in 1971 (Deacon and Williams 2010; Jimenez et al. 2017). Since that time, this species has been managed among numerous refuge habitats via assisted (re-) colonization to establish and/or re-establish refuge populations following local extirpation. Notably, two such extirpations followed the establishment of invasive species (Jimenzez et al. 2017; Kevin Guadalupe, Nevada Department of Wildlife, unpublished). For example, the Lake Harriet poolfish population declined from over 10,000 fish to 161 individuals in 2016, shortly after the discovery of invasive red swamp crayfish and western mosquitofish in 2012, and 2015, respectively. The population decline at Lake Harriet are consistent with a mesocosm study conducted by Goodchild and Stockwell (2016), where they had found poolfish juvenile productivity to be diminished in the presence of mosquitofish (Goodchild and Stockwell 2016). However, that experimental study was conducted using mesocosms with limited spatial structure, begging the question if poolfish may co-persist with mosquitofish under different circumstances.

We conducted three studies to evaluate mosquitofish impacts on Pahrump poolfish populations, specifically to test if impacts were repeatable amongst different experimental designs. First, we modeled the vulnerability of poolfish juveniles to gape-limited predation for two mosquitofish populations that differed in gape sizes. We then compared the impacts of these same two mosquitofish populations on juvenile survival of experimental populations of Pahrump poolfish. Finally, we replicated work reported by Goodchild and Stockwell (2016), however we altered the procedures by increasing the spatial complexity within the mesocosms to see if habitat complexity alters mosquitofish impacts on experimental poolfish populations.

2.3. Methods

2.3.1. Vulnerability Modeling

In order to measure the gape sizes of two distinct populations of mosquitofish we collected and sacrificed 200 fish from each western mosquitofish population: Garrett Spring (Garrett), Pershing Co. and Wabuska Spring (Wabuska), Lyon Co., Nevada. Previous work showed that these two populations were divergent in body size despite having shared common ancestry about 55 years earlier (Stockwell and Weeks 1999; Stockwell and Vinyard 2000). We measured gape-size of each mosquitofish from both populations to build prey vulnerability models foreach mosquitofish population. These models were based on relative prey vulnerability with associated body depths, where body depth is a measure of the widest part of a fish (Hambright et al. 1991). Prey vulnerability models assume that gape-size plays a large role in governing risk for prey items consumed by mosquitofish (Henkanaththegedara and Stockwell 2013). Prey with body depths exceeding the gape-size of the largest mosquitofish were considered unavailable to mosquitofish predation, while prey with body depths smaller than the smallest mosquitofish gape-size were considered vulnerable to predation by mosquitofish (Henkanaththegedara and Stockwell 2013).

2.3.2. General Mesocosm Methods

Experimental fish communities were established in circular 1211-liter rigid plastic tanks on North Dakota State University's Agricultural Experiment Station in Fargo, ND. Mesocosms were set up with approximately 950-l of dechlorinated water. Water levels were maintained through additions of water. Each tank contained one cubic foot of river rock (1-1.5 inches in diameter) as well as artificial cover material (Five 0.5 m-long clumps of plastic mesh weighted to simulate aquatic plants) added uniformly in all tanks to create breeding substrate. The mesocosms were inoculated with a mixture of plankton from a local semi-permanent wetland using a 500-micron dipnet after mixing the sediment to ensure benthic invertebrates were included within the sampling process. All tanks were aerated with an air stone to maintain dissolved oxygen of around 8-9mg/L. Water temperatures changed relative to environmental conditions in a diel rhythm and over the course of both experiments. Tanks were checked daily for deceased fish and to assure continuous airflow. Regular water quality tests revealed non-stressful conditions for dissolved oxygen, ammonia, and nitrate levels.

The number of fish initially stocked in each mesocosm differed between the two experiments due to year to year variation in fish available. For instance, very few male mosquitofish were captured in 2014, thus we used only gravid female mosquitofish. This was not a limitation because mosquitofish females store sperm (Medlen, 1951) and can give birth to separate broods every three to four weeks (Krumholtz 1948). All mesocosms received supplemental food to meet IACUC requirements. General experimental conditions for Goodchild and Stockwell (2016) were relatively similar, but some parameters varied such as structural cover and the number and composition of fish used. These changes are specified for each experiment below and summarized in Table 2.1.

2.3.3. Statistics

All data collected were analyzed using JMP Pro 14 software. We examined the responses of each species separately to test how sympatry affected juvenile production for each species. Due to the small sample sizes we used non-parametric Wilcoxon comparison of means to test for significant treatment effects on juvenile productivity. When necessary, a post-hoc Wilcoxon-each pair test was used which maintained experimental-wise error rate at an alpha of 0.05.

2.3.4. Experiment 1. An ex situ analysis of the effects on Pahrump poolfish by two distinct source populations of mosquitofish with varying associated in gape sizes

Poolfish were caught from Lake Harriet in Clark County, Nevada a habitat where poolfish were historically the only species, whereas mosquitofish were caught from the Garrett and Wabuska mosquitofish populations previously described (see Stockwell and Vinyard 2000). All fish were shipped live over-night to Fargo, ND. We established nine replicates of the following experimental communities: I) allopatric poolfish, II) poolfish sympatric with Garrett mosquitofish, and III) poolfish sympatric with Wabuska mosquitofish. To obtain additional insights on responses of mosquitofish, we established three replicates: *IV*) allopatric Garrett mosquitofish and *v*) allopatric Wabuska mosquitofish. Tanks were established with nine adult poolfish and/or nine gravid female mosquitofish. The initial poolfish sex ratio was unknown as poolfish are sexually monomorphic. Because mosquitofish males were exceptionally rare in our collections, we used only gravid females. This scenario was also matched the conditions for our vulnerability models which only included mosquitofish females, as females are larger due to sexual dimorphism.

Poolfish were stocked 11 days prior to mosquitofish to allow poolfish eggs to be laid and incubated. The experiment was terminated after approximately 7 weeks, at which time all tanks were seined to depletion. Adult poolfish adult survivorship was calculated on a per tank basis. Juveniles were counted on a per tank basis, while standard length was not assessed to minimize handling time.

2.3.5. Experiment 2. An ex situ analysis of Western mosquitofish effects on Pahrump poolfish adult survivorship and juvenile production within spatially complex habitats

During 2017, all mesocosms were provided with reclaimed PVC vinyl structures Fishiding® to simulate aquatic plants and to increase spatial structure. These habitats structures were not used in the previous mesocosm experiment (Goodchild and Stockwell 2016), nor in Experiment 1 (above). The spatial structure for the current study was measured using rugosity to ensure increased complexity relative to the previous study conducted by Goodchild and Stockwell (2016). Rugosity was measured following the protocol by Risk (1972), where a flexible metal chain was draped across the span of the mesocosm, keeping the chain aligned with the diameter of the cattle tank. The chain length required to span the cattle tank with structure present was then divided by the length of the mesocosm without the presence of structure.

The added structure was considered a procedural change to provide more cover for poolfish juveniles both in allopatric and sympatry with mosquitofish (Goodchild 2015; Goodchild and Stockwell 2016). We chose not to include treatments with poor spatial structure due to the limited number of experimental poolfish. Also, it was hard to justify including experimental conditions that were previously shown to be unsuitable for co-persistence of poolfish with mosquitofish.

Western mosquitofish were obtained from Sutter-Yuba Mosquito and Vector Control district in Yuba City, CA. Poolfish used in this experiment included wild poolfish collected from Shoshone Stock Pond (White Pine County, NV) on June 13, 2017, and lab-reared poolfish which were descended from poolfish originally collected in 2014 from Lake Harriet, Clark County (Goodchild and Stockwell 2016).

Fish communities included allopatric and sympatric populations of poolfish and mosquitofish. Eight individuals of the given species were used for each mesocosm, with sympatric treatments containing sixteen total fish. This experiment followed an additive approach where Fausch (1998) described this as the model for testing novel interactions following species invasions. While a substitutive design would have called for twice as many poolfish, which would minimize sample size due to limited availability of poolfish. For mosquitofish treatments each mesocosm received six females and two males, while poolfish received eight fish as we expected a female biased ratio concordant with typical observations for wild poolfish populations (Heckmann, 2009; Goodchild 2016). We established eight replicates of the following experimental communities: I) allopatric poolfish, II) allopatric mosquitofish, and III) poolfish sympatric with mosquitofish. The treatments were then randomly assigned within each of eight blocks for a total of 24 experimental tanks.

Supplemental food was provided every other day within each tank at rates of 5% of total fish biomass, as well as a once a week addition of brine shrimp (in compliance with IACUC#A15072). At 10 weeks, the experiment was terminated, and each tank was seined to depletion. Adult poolfish from each tank were weighed, measured for standard length, and adult survivorship was calculated on a per tank basis. Juveniles were weighed simultaneously to limit handling times with individual juveniles.

Table 2.1. Differing factors within three mesocosm designs for Pahrump poolfish and Western mosquitofish interactions conducted amongst three experimental designs; limited habitat complexity, variation amongst predator associated gape size, and increased spatial complexity.

	Goodchild and Stockwell 2016	Experiment #1 –population-specific impacts	Experiment #2 – Enhanced Spatial Structure
Enhanced Spatial Structure	Limited Complexity (No PVC structures)	Limited Complexity (No PVC structures)	Increased Spatial Complexity (3 PVC structures included)
Number of poolfish per tank and Source Population	9 Adult poolfish from Lake Harriet	9 Adult poolfish from Lake Harriet	8 Adult poolfish Lab Reared and Shoshone Stock Pond
Mosquitofish Founders	6 Females and 3 Males	9 Gravid Females*	6 Females and 2 Males
Number of Replicates	10	9	8
Percent Biomass Food per tank - per	5% Daily	5% Daily	5% Every other day
Experiment Set-up and Maintenance	Goodchild	Goodchild	Paulson
Experiment Duration (days)	71	49	70

* During collecting male mosquitofish were exceptionally rare, but the experiment was initiated with gravid females. The exclusion of males during this study does not impact the study, as females are capable of sperm storage and have rapid reproductive cycles not giving an inherent advantage being gravid at the start.

2.4. Results

2.4.1. Prey vulnerability modeling

Based on the 200 mosquitofish voucher samples from each source population, Garrett females had significantly larger gape sizes (3.19 ± 0.024; mean ± standard error) compared to Wabuska females (2.54 ± 0.024; F = 356.32, P < 0.0001). These data were used to model prey vulnerability as a function of prey body depth relative to the predator gape-size frequency distributions for each mosquitofish population (Hambright et al. 1991). The models produced two distinct curves for poolfish juvenile vulnerability regarding the two mosquitofish populations. Both populations of mosquitofish demonstrated a sharp decrease in prey vulnerability associated with a population specific gape-size threshold (Figure 2.1), for example poolfish juveniles that reached 2.1mm in body depth had similar expected vulnerabilities to predation by the two populations; 1.0 and 0.96 when sympatric with Garrett and Wabuska mosquitofish, respectively. However, by the time poolfish juveniles grow to a body depth of 2.8 mm the vulnerabilities are quite different; approximately 0.90 and 0.28 when sympatric with Garrett and Wabuska mosquitofish, respectively (Figure 2.1). Therefore, the model predicts that Wabuska fish should be less capable of consuming larger juveniles in comparison to the larger-bodied Garrett mosquitofish.

2.4.2. Experiment 1: An ex situ analysis of the effects on Pahrump poolfish by two distinct source populations of mosquitofish with varying associated in gape sizes

The mesocosms appeared to provide adequate environmental conditions, as allopatric populations had high adult survival and high juvenile production. Adult survival did not differ when comparing allopatric poolfish populations (100%) to poolfish populations sympatric with Garrett mosquitofish (97.5± 0.01%; Z = -1.42; P = 0.31) or to populations sympatric with

Wabuska mosquitofish (98± 0.01%; Z = -0.68; P = 0.99; Figure 2.2). There was no significant difference in adult poolfish survival for the latter two treatments (Z = 0.55; P = 0.58; Figure 2.2).

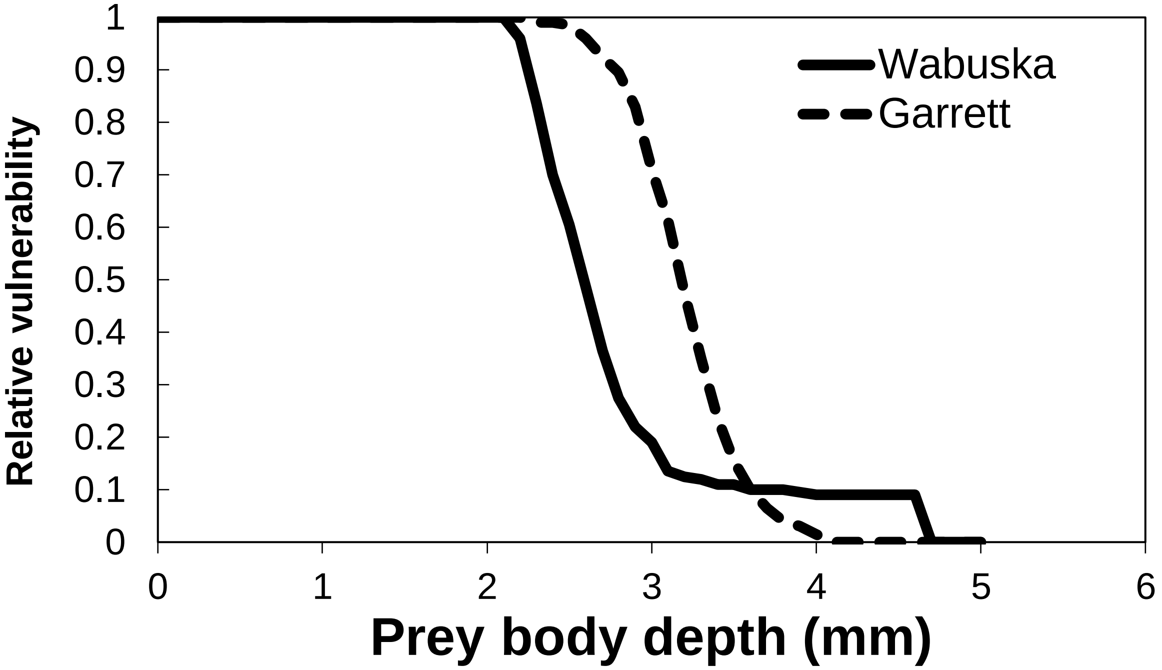

Figure 2.1. Prey vulnerability curves for Pahrump poolfish juveniles being consumed by two distinct phenotypes of western mosquitofish. Poolfish juvenile vulnerability to predation by large-bodied Garrett populations are represented by the dashed line and the solid black line depicts the vulnerability of poolfish predation by smaller-bodied Wabuska mosquitofish.

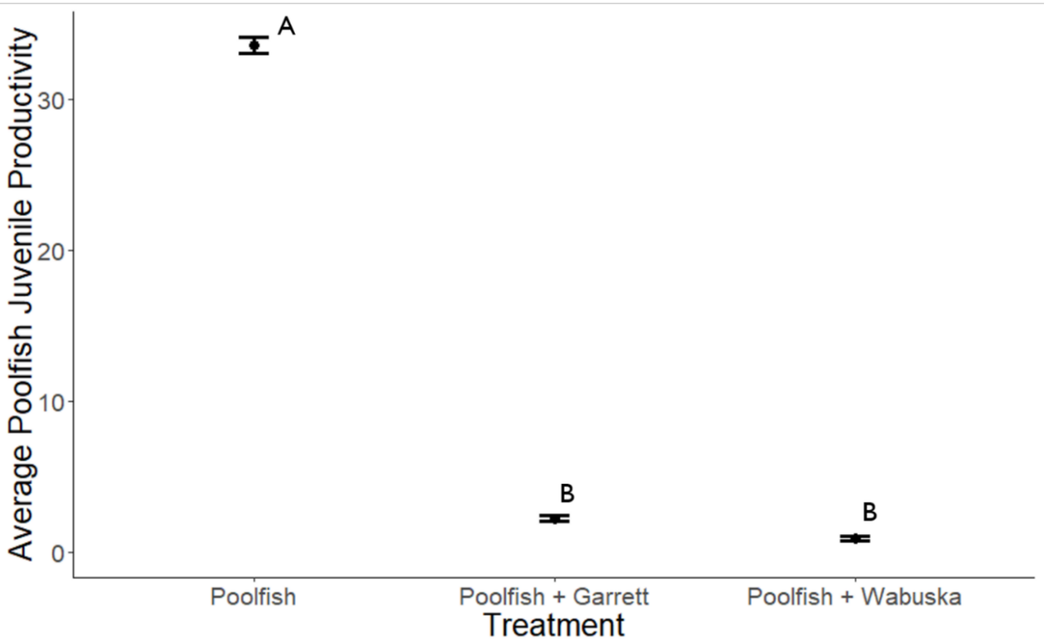

Figure 2.2. Average juvenile poolfish produced among nine replicates within limited spatial complexity for I.) allopatric poolfish, II.) sympatric poolfish and Garrett mosquitofish, and III.) sympatric poolfish and Wabuska mosquitofish. Treatments sharing a letter were not significantly different.

The number of poolfish juveniles per population differed significantly among treatments. There was significantly greater poolfish juvenile production when in allopatry (33.56 ±.53; mean number of juveniles/tanks ± standard error) than when sympatric with Garrett mosquitofish (2.22 ± 0.21; Z = 3.39; P = 0.0007) or when sympatric with Wabuska mosquitofish (0.89 ± .16; juveniles/tank; Z = 3.54; P = 0.0004; Figure 2.3). Poolfish juvenile production did not significantly differ between the two sympatric treatments (Z = -0.89; P = 0.37; Figure 2.3).

Poolfish impacts on production for both the Garrett and Wabuska populations of mosquitofish were not signficant, but the effect size differences were notable. For Garrett mosquitofish, there was no significant difference in juvenile production in allopatry (160.7 ± 33.0) compared to sympatry with poolfish (108 ± 13.8; Z=1.11; P=.26; Figure 2.4). Similarly, the number of Wabuska mosquitofish juveniles produced in allopatry (107 ± 29.2) was not significantly different from the number produced when sympatric with poolfish (65.56 ± 11.1; Z = 1.29; P = 0.19; Figure 2.4). Because brood size co-varies with mosquitofish female body size (Krumholz 1984; Palmquist and Stockwell 1995), we did not compare juvenile productivity between the two mosquitofish populations due to the population specific differences in body size differences.

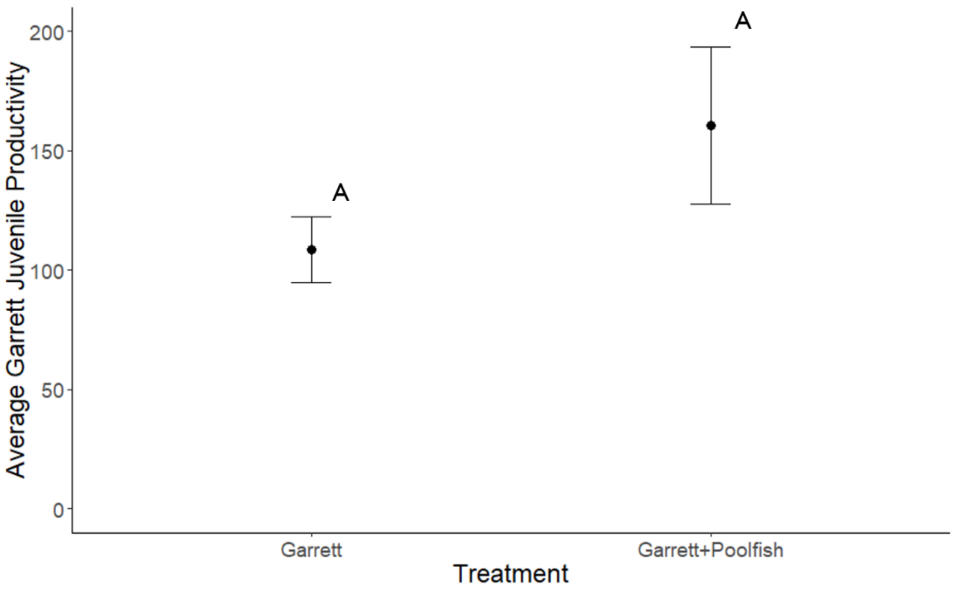

Figure 2.3. Average juvenile Garrett mosquitofish produced from nine replicates within limited spatial complexity for I) allopatric Garrett mosquitofish and II) sympatric Garrett mosquitofish and poolfish. Treatments sharing a letter were not significantly different.

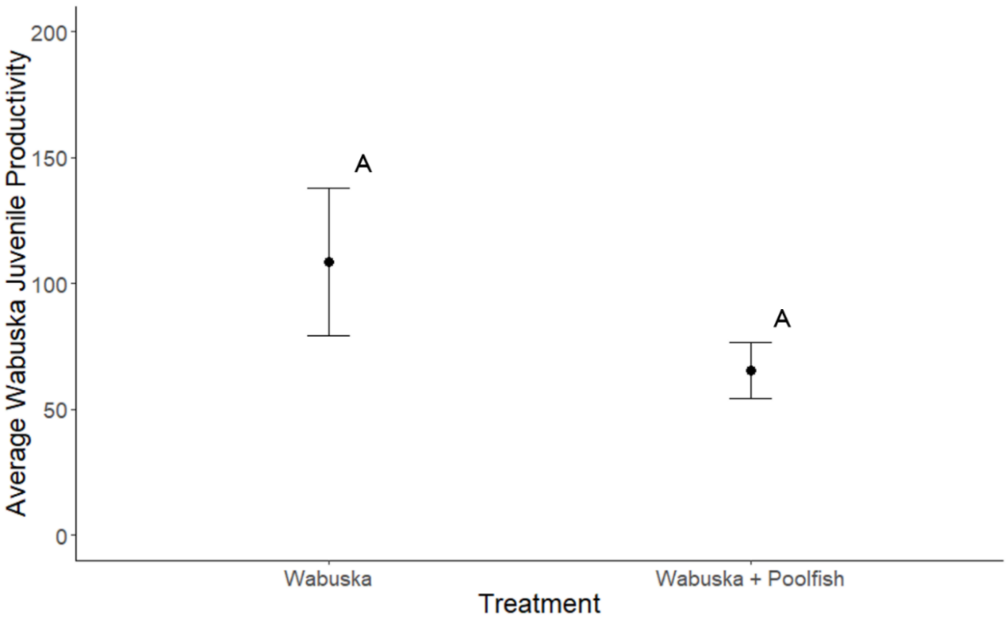

Figure 2.4. Average juvenile Wabuska mosquitofish produced from nine replicates within limited spatial complexity for I) allopatric Wabuska mosquitofish and II) sympatric Wabuska mosquitofish and poolfish. Treatments sharing a letter were not significantly different.

2.4.3. Experiment 2: An ex situ analysis of Western mosquitofish effects on Pahrump poolfish adult survivorship and juvenile production within spatially complex habitats

The mesocosms supported adequate environmental conditions for survival and reproduction as evidenced by low adult mortality and high juvenile production occurring within all allopatric mesocosms. Mortality did not significantly differ for adult poolfish when in allopatry (98 ± .07%; percentage survival ± standard error) compared to poolfish adult survival when sympatric with mosquitofish (87.5 ± 0.14%; Z = -1.66 P =.096).

Poolfish juvenile productivity was significantly higher for allopatric populations (77.6 ± 18.4 juveniles / tank± standard error) compared to poolfish productivity in the presence of mosquitofish (4.3 ± 0.9; Z = -3.32, P=0.0009; Fig. 2.5).

We were unable to determine adult mortality for mosquitofish, as it was not possible to differentiate between founder adults and F1 adults. However, mosquitofish juvenile production was not impacted by the presence of poolfish. Mosquitofish in allopatric tanks had produced on average similar amounts of juveniles (166.9±8.5) compared to mosquitofish productivity in sympatric tanks (149.3 ± 13.0; Z = -1.22, P = 0.23; Fig. 2.6).

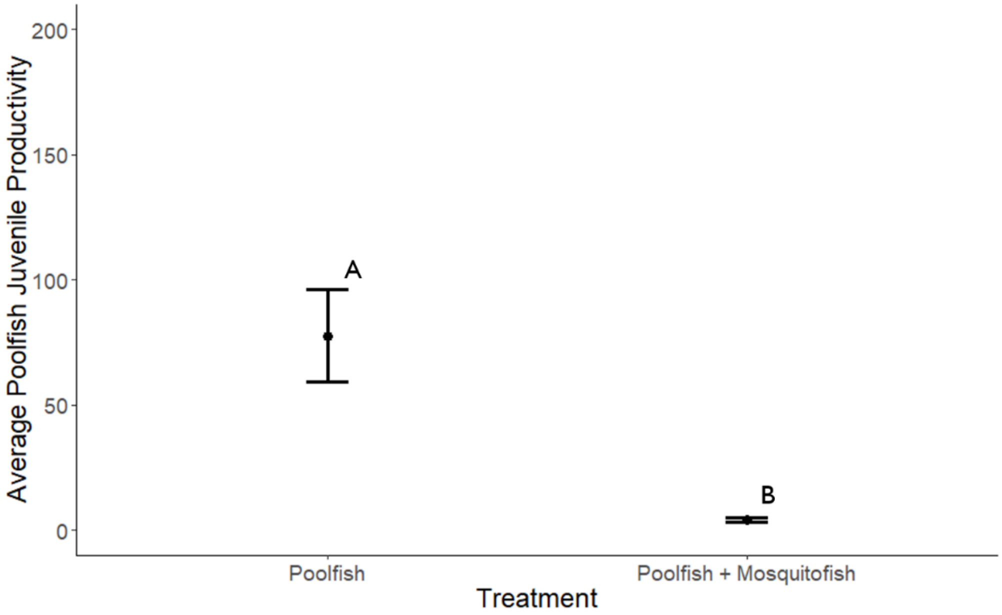

Figure 2.5. Average poolfish juvenile productivity among eight replicates within spatially complex habitats in both I) allopatry and II) sympatry with mosquitofish. Treatments sharing a letter were not significantly different.

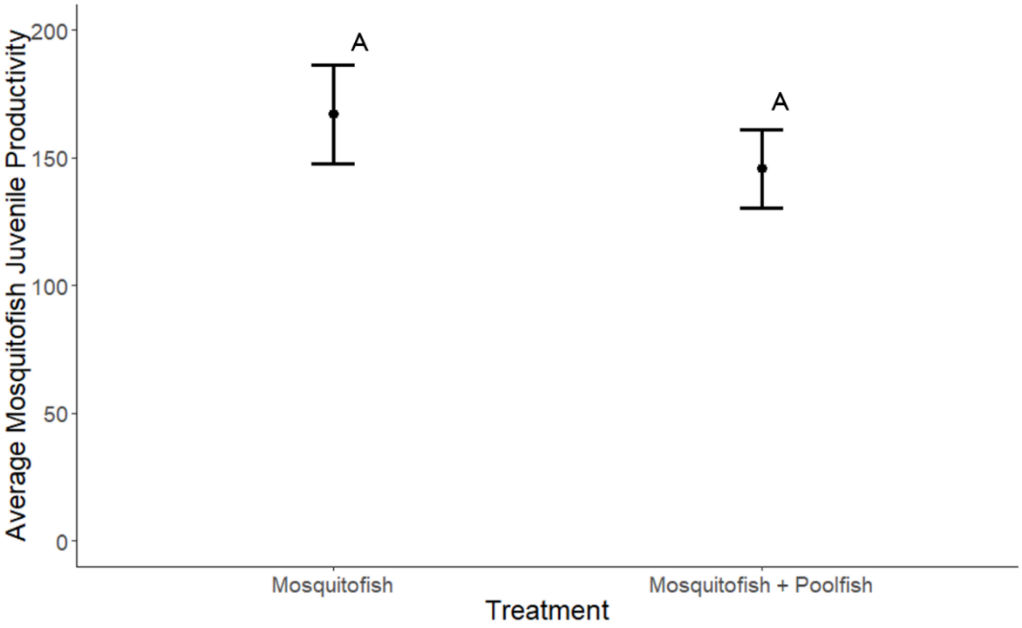

Figure 2.6. Average mosquitofish juvenile productivity among eight replicates within spatially complex habitats in both I) allopatry and II) sympatry with poolfish. Treatments sharing a letter were not significantly different.

2.5. Discussion

Desert fish species such as the endangered Pahrump poolfish are often managed using refuge habitats (Minckley 1995), but aquatic habitats in the desert are at a premium, and many potential habitats host invasive fish species (Miller, 1961; Minckley 1995; Jackson et al., 2001). These constraints motivated our research to explore possible contexts where Pahrump poolfish may co-persist with invasive mosquitofish. We considered contexts where poolfish might co-persistence with mosquitofish, as Goodchild and Stockwell (2015) demonstrated that within limited spatial mesocosms poolfish juvenile productivity was near zero.

First, we evaluated the relative vulnerability of poolfish juveniles to mosquitofish predation, based on the gape-size distributions for two phenotypically distinct populations of western mosquitofish. This case study focused on two populations of mosquitofish that differ in body sizes, and associated gape sizes. By the time poolfish juveniles reach a body depth of

2.8mm, the expected vulnerability to predation by larger-bodied Garrett mosquitofish are 0.96 with vulnerability dropping to 0.28 when sympatric with smaller-bodied Wabuska mosquitofish. These findings are consistent with recent work evaluating vulnerability of endangered Mohave tui chub (*Siphateles bicolor mojavensis*) to predation by western mosquitofish, where these researchers had found that once tui chub reached a certain body depth they no longer were vulnerable to predation (Henkanaththegedara and Stockwell 2013, 2014). Collectively, this model has produced the intuitive result that poolfish should have higher predation risk when sympatric with mosquitofish with larger gape-sizes.

Our vulnerability models predicted that poolfish juvenile production should be relatively higher when poolfish are sympatric with smaller-bodied Wabuska mosquitofish than when sympatric with the larger-bodied Garrett mosquitofish. However, there were virtually no poolfish juveniles produced when sympatric with either of the two mosquitofish populations. These findings suggest that poolfish juveniles did not grow fast enough to escape predation pressure exerted by either mosquitofish population. Unfortunately, no data are currently available regarding the scope of poolfish juvenile growth rates. However, another similar study demonstrated that razorback sucker larvae (*Xyrauchen texanus*) did not grow fast enough to escape predation pressure by invasive fish species (Marsh et al. 2011).

Within the second experiment, poolfish juvenile production was near zero even in habitats with increased relative spatial complexity compared to earlier work reported by Goodchild and Stockwell (2015). Our findings are contrary to numerous reports from marine systems where increased vegetative cover offered refugia to early life stage fish (Diehl, 1988; Menge and Lubchenco, 1981; Graham and Nash, 2013). Our cover type may not have been a suitable material for poolfish, as the Fishiding structures are generally used with species such as

Razorback sucker (*Xyrauchen texanus*). Poolfish larvae were regularly observed in the open water column away from structural cover, thus the increase in available cover may have not been fully utilized by the naïve species. By contrast, in a separate study, Amargosa pupfish larvae maintained closer proximity to cover in the benthos which may have contributed to the co-persistence of Amargosa pupfish with western mosquitofish (Goodchild and Stockwell 2016). It is also possible that co-persistence may be possible in heterogenous habitats that include both lentic and lotic characteristics. For instance, Scoppetone et al. (2005) reported that abundances and associated impacts of mosquitofish on the Ash Meadows Amargosa pupfish (*C. nevadensis mionectes*) were higher in lentic habitats. However, testing this hypothesis with poolfish would not be productive given that historic refuge habitats for Pahrump poolfish only include lentic habitats.

We recognize limitations of our work. For instance, we did not employ different levels of habitat complexity as a treatment factor. This decision was based on our limited number of mesocosms and available poolfish. Further, earlier work conducted in mesocosms with limited spatial structure (~30% lower complexity regarding measures of rugosity) showed that poolfish juvenile production was nearly zero in the presence of mosquitofish (Goodchild and Stockwell 2016). Thus, including such a treatment seemed a poor use of the limited number of experimental poolfish. It is possible that even higher levels of habitat complexity could enhance juvenile survival, but our anecdotal observations suggest the poolfish juveniles appeared to prefer the open water column despite the availability of structural complexity. We also recognize that competition may have also played a role in suppressing production of poolfish juveniles, however supplemental feeding likely limited resource competition, as feeding was relative to total biomass in each tank.

Finally, we employed an additive study design in which the total initial biomass of all adult fish was higher for the sympatric tanks. Fausch (1998) suggested that additive designs are to be used within studies with species that have differences in ecological niches, such as the case with pelagic poolfish and surface feeding mosquitofish (see Goodchild and Stockwell 2016). Additive designs were also suggested to be used when looking at the direct interaction between introduced species, which in our experiment mosquitofish are an introduced species. Further, a substitutive design would have required twice as many poolfish in the allopatric tanks compared to the sympatric tanks.

In conclusion, we saw repeatability negative impacts by mosquitofish on poolfish across experiments conducted under different contexts that included habitat complexity and variation in gape-limited predation pressure. Our work is consistent with limited observations where invasive species were associated with the extirpations of poolfish populations. Through repetitive measures across different experimental designs we have expanded our inference space and can state with reasonable confidence that the mere presence of mosquitofish is likely to have detrimental impacts on poolfish. Thus, this work confirms the stated concern of exotic fishes as a threat to poolfish recovery (USFWS, 1980). Furthermore, managers should continually monitor habitats for the introduction of invasive species and be prepared for aggressive efforts to remove invasive species whenever possible. For instance, the remaining Lake Harriet poolfish were rescued and transplanted to a hatchery while Lake Harriet was drained to eradicate invasive crayfish and mosquitofish. We suggest that such aggressive actions will be required for the continued persistence of the endangered Pahrump poolfish.

2.6. References

Ayala, J. R., R. B. Rader, M. C. Belk, and G. B. Schaalje. 2007. Ground-truthing the impact of invasive species: Spatio-temporal overlap between native least chub and introduced western mosquitofish. Biological Invasions 9:857-869.

Courtenay, W. R., and J.E. Deacon. 1983. Fish introductions in the American Southwest: a case history of Rogers Spring, Nevada. The Southwestern Naturalist, 28: 221–224.

Cox, J. G. and S. L. Lima. 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology and Evolution 21: 674-680.

Deacon, J. E., Minckley, W. L., and Udall, S. L. (1991). Battle against extinction: native fish management in the American West. Univ. Ariz. Press, Tucson, xviii, 517, 519-613.

Diehl, S. 1992. Fish predation and benthic community structure: The role of omnivory and habitat complexity. Ecology 73:1646-1661.

Dunson, W. A. and J. Travis. 1991. The role of abiotic factors in community organization. American Naturalist 138: 1067-1091.

Fausch, K. D. 1998. Interspecific competition and juvenile Atlantic salmon (*Salmo salar):* On testing effects and evaluating the evidence across scales. Canadian Journal of Fisheries and Aquatic Sciences 55:218-231.

Goodchild, S. C. (2015). Life History and Interspecific Co-Persistence of Native Imperiled Fishes in Single Species and Multi-Species Ex Situ Refuges. Ph.D. dissertation, North Dakota State University, Fargo, ND.

Goodchild, S. C., and Stockwell, C. A. (2016). An experimental test of novel ecological communities of imperiled and invasive species. Transactions of the American Fisheries Society, 145: 264-268.

Graham, N., and K. L. Nash. 2013. The importance of structural complexity in coral reef ecosystems. Coral Reefs 32(2):315-326.

Hambright, K. D. 1991. Experimental analysis of prey selection by largemouth bass: Role of predator mouth width and prey body depth. Transactions of the American Fisheries Society 120:500-508.

Henkanaththegedara, S. M. and C. A. Stockwell. 2013. The role of gape-limitation in intraguild predation between endangered Mohave tui chub and non-native western mosquitofish. Ecology of Freshwater Fish 22: 11–20.

Henkanaththegedara, S. M. and C. A. Stockwell. 2014. Intraguild predation may facilitate co-existence of native and non-native fish. Journal of Applied Ecology 51: 1057–1065.

Jones, G. P. 1986. Food availability affects growth in a coral reef fish. Oecologia 70:136-139.

Krumholz, L. A. 1948. Reproduction in the western mosquitofish, *Gambusia affinis* (Baird and Girard), and its use in mosquito control. Ecological Monographs 18: 1-43.

Laha, M., and H. T. Mattingly. 2006. Identifying environmental conditions to promote species coexistence: An example with the native Barrens topminnow and invasive western mosquitofish. Biological Invasions 8:719-725.

Littler, M. M., P. R. Taylor, and D. S. Littler. 1989. Complex interactions in the control of coral zonation on a Caribbean reef flat. Oecologia 80:331-340.

Lowe S. J., M. Browne and S. Boudjelas. 2000. 100 of the World's Worst Invasive Alien Species. IUCN/SSC Invasive Species Specialist Group (ISSG), Auckland, New Zealand.

Marsh, P. C. and D. R. Langhorst. 1988. Feeding and fate of wild razorback sucker. Environmental Biology of Fishes 21: 59-67.

Medlen, A. B. 1951. Preliminary observations on the effects of temperature and light upon reproduction in *Gambusia affinis*. Copeia 1951: 148-152.

Menge, B. A., and J. Lubchenco. 1981. Community organization in temperate and tropical rocky intertidal habitats: Prey refuges in relation to consumer pressure gradients. Ecological Monographs 51:429-450.

Meffe, G. K. 1985. Predation and species replacement in American southwestern fishes: A case study. Southwestern Naturalist 30: 175-187.

Miller, R.R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Miscellaneous Publications of the Museum of Zoology, University of Michigan 68:1-155.

Miller, R.R. 1961. Man and the changing fish fauna of the American Southwest. Pap. Mich. Acad. Sci. 46: 365–404.

Minckley, W. L., and Deacon, J. E. (1968). Southwestern Fishes and the Enigma of" Endangered Species". Science 159: 1424-1432.

Nilsson, P.A. and C. Bronmark. 2000. Prey vulnerability to a gape-size limited predator: behavioral and morphological impacts on Northern pike piscivory. Oikos 88: 539-546.

Ouyang, Xu, Jiancao Gao, Meifeng Xie, Binghua Liu, Linjun Zhou, Bojian Chen, Jonas Jourdan, Rüdiger Riesch, and Martin Plath. (2018). Natural and sexual selection drive multivariate phenotypic divergence along climatic gradients in an invasive fish. Scientific reports, 8: 11164.

Palkovacs, Eric P., Michael C. Marshall, Brad A. Lamphere, Benjamin R. Lynch, Dylan J. Weese, Douglas F. Fraser, David N. Reznick, Catherine M. Pringle, and Michael T. Kinnison. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364: 1617-1628.

Palmquist, D. E. and CA Stockwell 1995. Covariance analysis with a covariate interaction: an example of a simple linear regression comparison technique. Conference on Applied Statistics in Agriculture.

Pen, L. J., and I. C. Potter. 1991. Reproduction, growth and diet of *Gambusia holbrooki* Girard) in a temperate Australian river. Aquatic Conservation: Marine and Freshwater Ecosystems 1:159-172.

Reznick, DA, H. Bryga, and JA Endler. 1990. Experimentally-Induced Life-History Variation in a Natural Population. Nature 346: 357-359.

Risk, M. J. 1972. Fish diversity on a coral reef in the Virgin Islands. Atoll Research Bulletin 153: 1-6.

Schoenherr A.A. (1981) The role of competition in the replacement of native fishes by introduced species. In: Fishes in North American Deserts (Eds R.J. Naiman and D.L. Soltz), pp. 173–203. John Wiley and Sons, New York, NY

Schriver, P., J. Bogestrand, E. Jeppesen, and M. Sondergaard. 1995. Impact of submerged macrophytes on fish-zooplanl phytoplankton interactions: Large-scale enclosure experiments in a shallow eutrophic lake. Freshwater Biology 33: 255-270.

Scoppettone, G. G., P. H. Rissler, C. Gourley, and C. Martinez. 2005. Habitat restoration as a means of controlling non-native fish in a Mojave Desert oasis. Restoration Ecology 13:247-256.

Selby, D. A. 1977. Thermal ecology of the Pahrump killifish, Empetrichthys latos Miller. Master Thesis, University of Nevada, Las Vegas, May 1977.

Sheffer, R. J., P. W. Hedrick, W. L. Minckley, and A. L. Velasco. 1997. Fitness in the endangered Gila topminnow. Conservation Biology 11:162-171.

Simons, L. H., D. A. Hendrickson, and D. Papoulias. 1989. Recovery of the Gila topminnow: A success story? Conservation Biology 3:11-15.

Stearns, S. C. 1983. A natural experiment in life-history evolution: field data on the introduction of mosquitofish (Gambusia affinis) to Hawaii. Evolution 1983: 601-617.

Stockwell, C. A., and Vinyard, G. L. 2000. Life history variation in recently established populations of western mosquitofish (*Gambusia affinis*). Western North American Naturalist. 2000: 273-280.

Stockwell, C. A. and Weeks, S. C. 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). Animal Conservation, 2: 103–110.

Sumpter, D. J. (2006). The principles of collective animal behavior. Philosophical Transactions of the Royal Society B: Biological Sciences, 36: 5-22.

USFWS (U.S. Fish and Wildlife Service). 1980. Pahrump killifish recovery plan. March 17, 1980. Washington, D. C. 30pp.

Winemiller, K. O., and A. A. Anderson. 1997. Response of endangered desert fish populations to a constructed refuge. Restoration Ecology 5:204-213.

CHAPTER 3. THE COMBINED EFFECTS OF MULTIPLE INVASIVE SPECIES ON IMPERILED PAHRUMP POOLFISH POPULATION PERSISTENCE

3.1. Abstract

Invasive species impacts are generally studied on a single-species basis; however, many systems have been invaded by numerous invasive species. We evaluated population level responses of the endangered Pahrump poolfish (*Empetrhichys latos latos*) to two common invasive species; western mosquitofish (*Gambusia affinis*) and red swamp crayfish (*Procambarus clarkii*). This study mimicked the recent introduction of crayfish and mosquitofish to a poolfish refuge habitat, Lake Harriet, Nevada. We used mesocosms that included experimental poolfish population sympatric with crayfish, and a treatment containing sympatric poolfish, crayfish, and mosquitofish.

We found that allopatric poolfish adult survival (95.6%±.03%; percentage adult survival ± standard error) was significantly higher than either treatment containing crayfish (x^2= 86.33, p=0.0001), where treatments containing poolfish sympatric with crayfish and treatments with poolfish sympatric with both crayfish and mosquitofish did not differ (z=.33; p=0.74). Allopatric poolfish juvenile productivity (91.43±11.98; juveniles± standard error) did not differ when compared to poolfish sympatric with crayfish (64.85±19.02; z=-1.28; p=0.20). Allopatric juvenile productivity was only impacted when mosquitofish were present (1.85±.51)(z=-3.58; p=0.001). These results suggest that crayfish impact adult survivorship, while mosquitofish impact juvenile productivity.

3.2. Introduction

The introduction of invasive species has become so widespread that many systems have been invaded by multiple invasive species (Garcia-Berthou, 2005; Gallardo et al., 2016). Thus, the combined effects of multiple species introductions on native species is an important topic of concern to conservation biologists (Pyšek and Richardson, 2010; Ricciardi et al., 2013). Most empirical work has focused on impacts by only one invasive species, however there are a few studies have shown that multiple invasive species can have substantial impacts on endemic species. For example, Dénes et al., (2018) demonstrated the additive impacts of invasive cattle, feral pigs, and wild horses, of which directly lowered seed abundance of the endangered Paraná pine (*Araucaria angustifolia*). This effectively lowered food availability for ~70 endemic species, and ultimately lowered successful productivity of Paraná pine saplings (Dénes et al., 2018). Avian malaria is another example of multiple species introduction, where the introduction of malaria was facilitated by the establishment of *Plasmodium relictum* and the associated vector, Southern House mosquito (*Culex quinquefasciatus*). The spread of avian malaria was further exacerbated by feral pigs (*Sus scrofa*) forming cavities within trees through foraging behavior, furthermore increasing mosquito breeding habitat availability (LaPointe et al. 2012). The spread of avian malaria led to contractions of Hawaiian avifauna ranges to higher elevations where cooler temperatures exclude mosquitoes (Samuel et al., 2011; LaPointe et al., 2012).

In many aquatic ecosystems, particularly in freshwater estuaries there have been significant increases in the number of invasive species introduced, specifically through ballast water exchange (Carlton and Geller, 1993). Ballast water exchange resulted in the introduction of Eurasia zebra mussel (*Dreissena polymorpha*), and invasive fish, the Round goby (*Neogobius melanostomus*), these two species which have been shown to have combined impacts on aquatic communities (Jude, 1992; Jude and Jansen, 2001). Zebra mussels rapidly filter phytoplankton, altering the planktonic community structure. As the zooplankton community shifts through manipulation by zebra mussels the round goby is capable of outcompeting endemic species for the newly formed zooplankton community, resulting in the extirpation of the endemic species, such as the mottled sculpin (*Cottus bairdii*) (Jansen and Jude, 2001).

Invasive species are a major concern for various desert springs in the southwestern United States, where invasive species have been directly associated with the decline and extirpation of many endemic aquatic species (Minckley and Deacon, 1968; Soltz and Naiman 1978; Cucherousset, and Olden, 2011). In several cases, numerous invasive species have co-invaded habitats within the desert southwest. For example, numerous poecilid species invaded springs in southern Nevada (La Rivers 1962; Deacon et al. 1964). Furthermore, invasive macroinvertebrates such as red swamp crayfish were introduced into the southwest for commercial production, and likely dispersed to non-commercial water-bodies from an aquaculture facility (Deacon et al., 1964; Miller, 1989; Deacon and Williams, 1991; Paulson and Martin, 2014).

These introductions were often associated with the concurrent decline of native fishes (Deacon et al. 1964). The impacts of invasive species within desert ecosystems have been attributed to predator naïveté of endemic fishes, which evolved in depauperate communities. Specifically, endemic fishes are hypothesized to have lost anti-predator traits as they evolved in allopatry with limited predation and competition, thus making them vulnerable to invasive predators (Miller 1961; Minckley and Deacon 1968; Courtenay and Deacon 1983; Cox and Lima 2006), such as the western mosquitofish (*Gambusia affinis*) and red swamp crayfish (*Procambarus clarkii*).

Western mosquitofish were introduced to California in 1922 and subsequently to Nevada in 1934 (La Rivers, 1994; Stockwell et al., 1996), while red swamp crayfish were used commonly in aquaculture in the 1930's and first documented invading the Ash Meadows spring system in 1942. Both species can have rapid population growth rates and broad ecological tolerances (Huner and Lindqvist, 1995; Paulson and Martin, 2014), resulting in the ability to have substantial impacts on native fishes (Miller, 1989; Meffe et al., 1985). Mosquitofish are voracious predators of fish eggs and larvae (Pyke, 2008), while crayfish prey on the adults and larvae of benthic fishes (Taylor and Thomas 2013). For example, the decline of the Gila topminnow (*Poeciliopsis occidentalis*) corresponded with the invasion of western mosquitofish and habitat degradation (Miller, 1961; Minckley and Deacon, 1968; Meffe et al., 1985). Similarly, red swamp crayfish presumably contributed to the extinction of the Ash Meadows killifish (*Empetrichthys merriami*) (Miller, 1989).

The direct effects of both red swamp crayfish and western mosquitofish have been independently evaluated amongst numerous experimental studies (Mills et al. 2004; Rogowski and Stockwell 2006; Thomas and Taylor, 2013; Goodchild and Stockwell 2016, Chapter Two), however the possibility for combined effects have not yet been empirically studied. Rogowski and Stockwell (2006) showed that experimental populations of White Sands pupfish (*Cyprinodon tularosa*) declined when sympatric with Virile crayfish (*Orconectes virilis*) at high densities or when sympatric with mosquitofish. Rogowski and Stockwell (2006), did not test for the combined effects of crayfish and mosquitofish on pupfish population growth.

Understanding the possible interactions of both western mosquitofish and red swamp crayfish is useful as both species are capable of rapidly spreading throughout aquatic systems in the southwestern U.S. For example, the impacts of multiple invasive species are listed as the greatest threat to the various endemic fishes within Ash Meadows of southern Nevada (Sada, 1990). The co-invasion of red swamp crayfish and western mosquitofish have been associated with the decline of two refuge populations of Pahrump poolfish. In fact, the largest refuge population of the endangered Pahrump poolfish (*Empetrichthys* latos latos) at Lake Harriet rapidly declined following the sequential colonization of red swamp crayfish in 2012, followed by the discovery of western mosquitofish in 2015. The poolfish population declined from over 10,000 fish in 2015 to 161 fish the following year (Kevin Guadalupe Nevada Department of Wildlife Report, 2016). when the remaining poolfish were salvaged and relocated to a fish hatchery (Guadalupe, 2016).

This population decline at Lake Harriet inspired us to use an experimental approach to evaluate the combined effects of crayfish and mosquitofish on experimental poolfish populations. Specifically, this research focused on sympatric relationships of poolfish, crayfish and mosquitofish to replicate the co-invasion of these two species at Lake Harriet. This study directly tested the combined effects of dual species invasion on the Pahrump poolfish populations.

3.3. Methods

Western mosquitofish were obtained from Sutter-Yuba Mosquito and Vector Control district in Yuba City, CA. Poolfish used in this experiment included wild poolfish collected from Shoshone Stock Pond (White Pine County, NV) on June 13, 2017, and lab-reared poolfish which were descended from poolfish originally collected in 2014 from Spring Mountain Ranch State Park, Clark County (Goodchild and Stockwell 2016). Red swamp crayfish were sourced from Carolina Biological Suppliers, Burlington, NC.

There were three treatments: I.) allopatric poolfish, II.) poolfish sympatric with crayfish, and III.) poolfish sympatric with both mosquitofish and crayfish. We did not include a poolfish + mosquitofish treatment because three previous experiments consistently showed poolfish juvenile production was nearly zero when mosquitofish were present (Goodchild and Stockwell 2016, Chapter 2). We utilized these treatments as a replication for the invasions of Lake Harriet in both 2012 and 2015, hypothesizing that there were likely combined effects of dual invasion.

Each block was replicated seven times for a total of 21 experimental tanks. All 21 tanks received seven poolfish of indeterminate sex. Four individual crayfish were introduced into two randomly selected mesocosms per block. One of the two crayfish mesocosms within each block was randomly selected to receive mosquitofish, including; five gravid females and two males. Crayfish density was maintained by replacing any crayfish that periodically died. Mortality was assessed by visual inspection daily.

All mesocosms were provided with three reclaimed PVC vinyl structures Fishiding® to simulate aquatic plants and to increase spatial structure along with two cubic feet of river rock, to simulate a more natural environment within the mesocosms. Supplemental food was provided every day within each tank at rates of ~2-3% of total fish biomass. We also provided Aquatic Arts sinking pellets as a supplemental food source for crayfish twice weekly at rates ~5% of the overall crayfish biomass. Water quality was assessed weekly for ammonia and nitrates. All tanks were checked daily, looking for mortalities for both species, and to assure air flow was constant from air stones.

At ten-weeks, the tanks were drained and seined to completion. Adult survivorship was calculated on a per tank basis. Juveniles were enumerated on a per tank basis as a measure of productivity within each mesocosm. No crayfish juveniles were observed within the experiment.

Mosquitofish adult survival was near 100% and juvenile production was substantial (286.57±26.72 mosquitofish juveniles per tank)

All data collected were analyzed used JMP Pro 14 software. Non-parametric Wilcoxon comparison of means were used to determine statistical differences between treatments, utilizing a block design. For comparisons among all treatments, post-hoc non-parametric Wilcoxon pairwise comparisons were conducted with experimental-wise alpha level of 0.05.

3.4. Results

In allopatry, adult poolfish survival rates were near 100% (95.6%±.03%; Survival percentage± standard error) and significantly higher than the sympatric treatments (x^2= 86.33, p=.0001). When compared to allopatric poolfish populations, survival was significantly lower both for the poolfish sympatric with crayfish (53.1%±.16%; z=-3.2; p=.0035) and the poolfish sympatric with both crayfish, and mosquitofish (55.1% ±.21%; z=-2,98; p=.0086; Figure 3.1). The latter two treatments did not significantly differ from each other in their effects on poolfish adult survivorship (z=.33; p=.74).

Juvenile productivity rates were significantly different among the three treatments (x^2= 14.2; p=.0008). Allopatric poolfish juvenile productivity (91.43±11.98) did not differ when compared to poolfish sympatric with crayfish (64.85±19.02; z=-1.28; p=.20). However, for the treatment containing all three species, poolfish productivity was significantly decreased (1.85±.51) when compared to the allopatric poolfish (z=-3.58; p=.001) and when compared to poolfish juvenile production when sympatric with only crayfish (z=-3.08; p=.002; Figure 3.2).

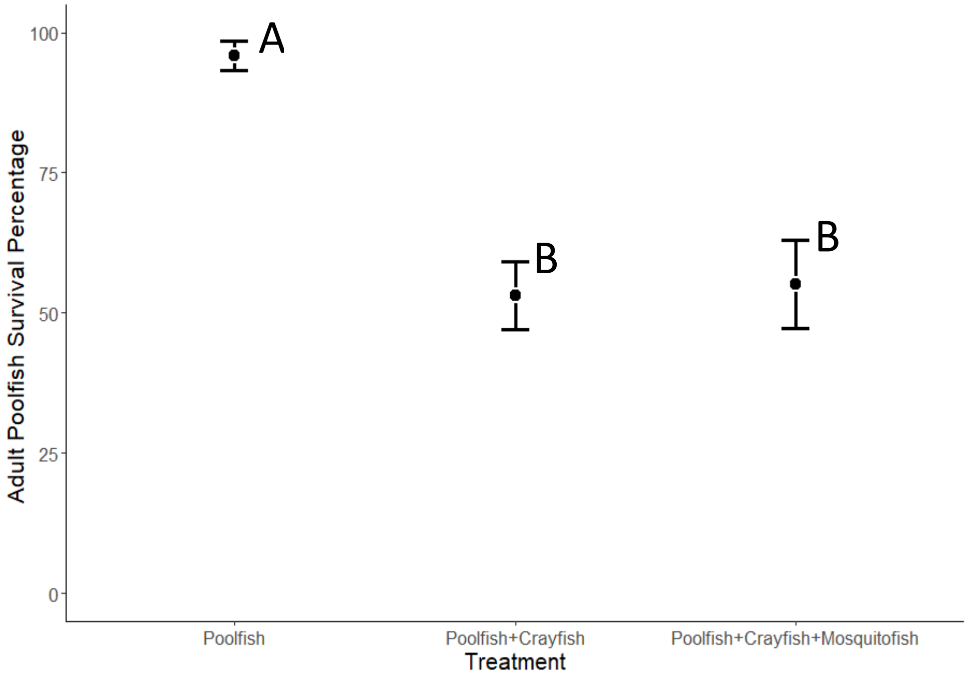

Figure 3.1. Adult poolfish survival averaged across seven replicates are shown for poolfish populations in I) allopatric populations as well as II) poolfish populations sympatric with crayfish and III) poolfish populations sympatric with both crayfish and mosquitofish. Treatments sharing a letter were not significantly different.

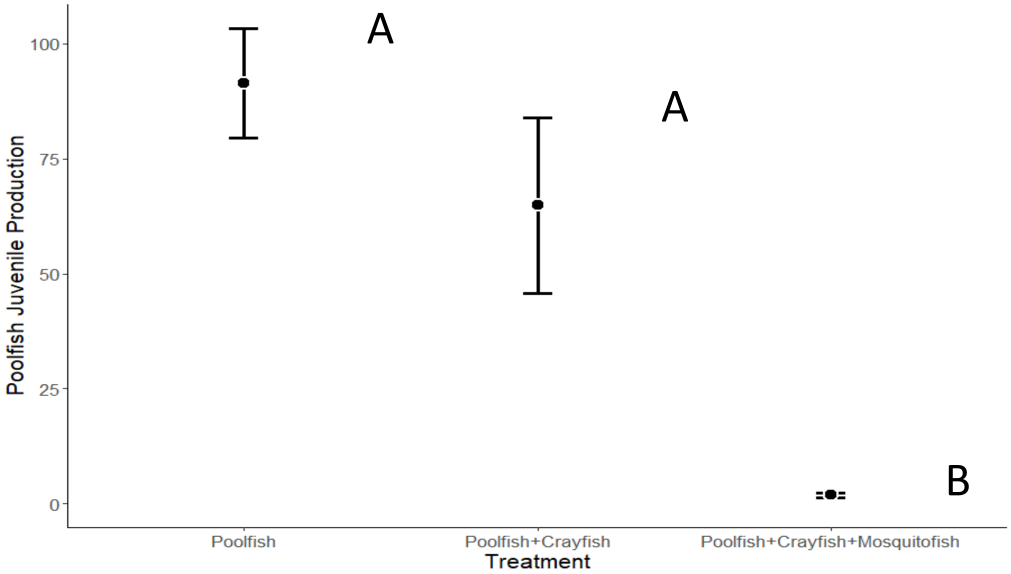

Figure 3.2. Juvenile productivity averaged across seven replicates are shown for poolfish populations in I) allopatry, as well as I) poolfish populations sympatric with crayfish and II) poolfish populations sympatric with both crayfish and mosquitofish. Treatments sharing a letter were not significantly different.

3.5. Discussion

Numerous mesocosm and observational studies have focused on the effects of specific invasive species. For instance, many studies have shown that mosquitofish have significant impacts on juvenile production of native desert fishes (Rogowski and Stockwell, 2006; Goodchild, 2016; Chapter 2). However, there have been limited efforts to evaluate the combined effects of multiple invasive species such as the western mosquitofish and red swamp crayfish.

In our study, adult poolfish mortality was significantly higher for treatments including crayfish, but the addition of mosquitofish did not have any additive effects on poolfish adult survival (Figure 3.1). The presence of crayfish alone resulted in a non-significant but 30% reduction in poolfish juvenile production while the combined effects of both crayfish and mosquitofish severely impacted poolfish juvenile production.

Our findings suggest that the introduction of crayfish may have notable impacts on the survival of poolfish adults, however poolfish populations are likely to persist as impacts on adult survivorship may be mediated by substantial juvenile production. Thus, crayfish are unlikely to have immediate acute impacts, rather long-term impacts may be more probable, as our overall juvenile production values were large enough to sustain population growth. This hypothesis is supported by empirical observation of long-term co-persistence of Pahrump poolfish and red swamp crayfish (8 years) within the Corn Creek refuge habitat (Kevin Guadalupe NDOW Report, 2016). Furthermore, the Lake Harriet poolfish population displayed an initial decline in abundance, however for three years after the discovery of red swamp crayfish the poolfish population was relatively stable at approximately 10,000 individuals (Guadalupe 2016). Nevertheless, within one year of mosquitofish invasion, the Lake Harriet poolfish population declined from more than 10,000 fish to less than 200 fish (Guadalupe, 2016). These findings are consistent with earlier work showing severe impacts of mosquitofish on juvenile productivity (Goodchild, 2012; 2016; Chapter 2).

Our work has relevance for understanding historic impacts of invasive species on several endemic species within Ash Meadows. For example, Miller (1989) attributed extinction of the Ash Meadows killifish (*Empetrichthys merriami)* to crayfish, while Minckley and Deacon (1968) inferred that Ash Meadows killifish extinction occurred following the invasion of multiple invasive species. The more recent decline of Warm Springs pupfish (*Cyprinodon nevadensis pectoralis*) was attributed to the combination of crayfish and mosquitofish (Sada, 1990). However, it is notable that the Ash Meadows Amargosa pupfish (*C. n. mionectes*) persisted with both invasive mosquitofish and crayfish at various springs in Ash Meadows. Scoppetone et al. (2005) hypothesized that habitat segregation associated with spatial variability in temperature may have facilitated co-persistence of pupfish with mosquitofish (Scoppetone et al., 2005). Collectively, these observations combined with our experimental data suggest that the extinction of *E. merriami* within Ash Meadows may have been due to more than the solitary impacts of red swamp crayfish. It is notable that poolfish were historically very rare based on historic collections at Ash Meadows (Miller, 1961).

Overall this study combined with previous mesocosm experiments have demonstrated that Pahrump poolfish are severely impacted by the presence of invasive species (Goodchild 2012, 2016; Paulson and Stockwell, In Prep). Thus, the current approach of managing Pahrump poolfish in single species refugia is clearly warranted. Crayfish did not severely hinder juvenile productivity, thus poolfish populations may co-persist with crayfish at least over the short-term. As our experiment was conducted for only a 10-week period a long-term study may be needed to determine the annual effects crayfish may have on poolfish populations. In fact, poolfish have co-persisted with red swamp crayfish for at least 8 years at the Corn Creek Refuge (Kevin Guadalupe NDOW Report, 2016). However, immediate intervention should be taken if western mosquitofish invade any of the poolfish refuge habitats. Our study shows the value of evaluating the combined effects of multiple invasive species on native species, but additional work should be undertaken to evaluate other combinations of invasive species.

3.6. References

Cariton, J. T., and J. B. Geller. 1993. Ecological roulette: The global transport of nonindigenous marine organisms. Science 261:78-82.

Clavero, M., and E. García-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution 20:110.

Courtenay Jr, W. R., and J. E. Deacon. 1983. Fish introductions in the American southwest: A case history of Rogers Spring, Nevada. The Southwestern Naturalist 1983:221-224.

Cox, J. G., and S. L. Lima. 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology and Evolution 21:674-680.

Cucherousset, J., and J. D. Olden. 2011. Ecological impacts of nonnative freshwater fishes. Fisheries 36(5):215-230.

Deacon, J. E., C. Hubbs, and B. J. Zahuranec. 1964. Some effects of introduced fishes on the native fish fauna of southern Nevada. Copeia 1964:384-388.

Deacon, J. E., and C. D. Williams. 1991. Ash meadows and the legacy of the Devils hole pupfish. Battle Against Extinction: Native Fish Management in the American West 1991:69-87.

Dénes, F. V., J. L. Tella, V. Zulian, N. P. Prestes, J. Martínez, and F. Hiraldo. 2018. Combined impacts of multiple non-native mammals on two life stages of a critically endangered neotropical tree. Biological Invasions 20:3055-3068.

Gallardo, B., M. Clavero, M. I. Sánchez, and M. Vilà. 2016. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology 22:151-163.

Goodchild, S. C. 2016. Life History and Interspecific Co-Persistence of Native Imperiled Fishes in Single Species and Multi-Species Ex Situ Refuges. North Dakota State University 2016.

Guadalupe, K. 2016. Nevada Department of Wildlife Report. Unpublished.

Hobbs, H. H., J. P. Jass, and J. V. Huner. 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). Crustaceana 56:299-316.

Hobbs, H. H., and D. J. Zinn. 1948. Crayfish in southern Nevada. Science 107:369.

Janssen, J., and D. J. Jude. 2001. Productivity failure of mottled sculpin *Cottus bairdi* in calumet harbor, southern lake Michigan induced by the newly introduced round goby *Neogobius melanostomus*. Journal of Great Lakes Research 27:319-328.

Jude, D. J., R. H. Reider, and G. R. Smith. 1992. Establishment of Gobiidae in the Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 49:416-421.

LaPointe, D.A., Atkinson, C.T. and Samuel, M.D., 2012. Ecology and conservation biology of avian malaria. Annals of the New York Academy of Sciences, 1249:211-226.

La Rivers, I. 1962. Fish and fisheries of Nevada. Nevada State Fish and Game Commission. Carson City, NV .

Meffe, G. K. 1985. Predation and species replacement in American southwestern fishes: A case study. The Southwestern Naturalist 1985:173-187.

Miller, R. R. 1961. Man, and the changing fish fauna of the American southwest. Michigan Academy of Science, Arts, and Letters.

Miller, R. R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Michigan Academy of Science, Arts and Letters 68:1-200.

Mills, M. D., R. B. Rader, and M. C. Belk. 2004. Complex interactions between native and invasive fish: The simultaneous effects of multiple negative interactions. Oecologia 141(4):713-721.

Minckley, W. L., and J. E. Deacon. 1968. Southwestern fishes and the enigma of" endangered species". Science 159:1424-1432.

Paulson, E. L., and A. P. Martin. 2014. Discerning invasion history in an ephemerally connected system: Landscape genetics of *Procambarus clarkii* in ash meadows, Nevada. Biological Invasions 16:1719-1734.

Pyke, G. H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. Annual Review of Ecology, Evolution, and Systematics 39:171-191.

Pyšek, P., and D. M. Richardson. 2010. Invasive species, environmental change and management, and health. Annual Review of Environment and Resources 35:25-55.

Ricciardi, A., M. F. Hoopes, M. P. Marchetti, and J. L. Lockwood. 2013. Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs 83:263-282.

Sada, D. W. 1990. Recovery plan for the endangered and threatened species of Ash Meadows, Nevada. US Fish and Wildlife Service.

Samuel, M. D., P. H. Hobbelen, F. DeCastro, J. A. Ahumada, D. A. LaPointe, C. T. Atkinson, B. L. Woodworth, P. J. Hart, and D. C. Duffy. 2011. The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: A modeling approach. Ecological Applications 21:2960-2973.

Scoppettone, G. G., P. H. Rissler, C. Gourley, and C. Martinez. 2005. Habitat restoration as a means of controlling non-native fish in a Mojave Desert oasis. Restoration Ecology 13:247-256.

Soltz, D. L., and R. J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History of Los Angeles County.

Stockwell, C. A., M. Mulvey, and G. L. Vinyard. 1996. Translocations and the preservation of allelic diversity. Conservation Biology 10:1133-1141.

Thomas, C. L., and C. A. Taylor. 2013. Scavenger or predator? examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. Freshwater Science 32.4: 1309-1317.

Wiles, G. J., J. Bart, R. E. Beck Jr, and C. F. Aguon. 2003. Impacts of the brown tree snake: Patterns of decline and species persistence in Guam's avifauna. Conservation Biology 17:1350-1360.

CHAPTER 4. DENSITY DEPENDENT EFFECTS OF INVASIVE RED SWAMP CRAYFISH (*PROCAMBARUS CLARKII*) ON AMARGOSA PUPFISH (*CYPRINODON NEVADENSIS AMARGOSAE*) POPULATIONS

4.1. Abstract

It’s expected that the impacts of invasive species are proportional to their densities. The invasive Virile crayfish (*Orconectes virilism*) impacted the threatened White Sands pupfish (*Cyprinodon tularosa*) corresponding with increasing densities of crayfish within a mesocosm design (Rogowski and Stockwell, 2006). However, Thomas and Taylor (2013) reported that impacts on benthic fish only occurred when Signal crayfish (*Pacifastacus leniusculus)* were in low density compared to high-density. The larger impact within low density treatments was theorized to be due to intraspecific interference competition among crayfish. These contradictory and surprising findings between two similar studies inspired us to test density-specific crayfish impacts on the Amargosa pupfish (*Cyprinodon nevadensis amargosae*).

We found that crayfish impacts were dependent on density for pupfish. Both allopatric pupfish (704 + 77; average juveniles/treatment ± standard error) and pupfish sympatric with low-density crayfish (705±33) produced significantly more juveniles than pupfish sympatric with high-density crayfish (271 ±36 z=3.31; p=.0009; and z=3.31; p=.0009, respectively). Tethering of high-density crayfish produced the opposite outcome predicted by Thomas and Taylor (2013). For the two high-density crayfish treatments, tethering caused a significant increase in juvenile production (493±94) compared to the high-density untethered crayfish (271±36) (z=2.257; p=.026). These findings suggest, reducing crayfish density will be beneficial for pupfish population persistence.

4.2. Introduction

The introduction of invasive species is a major driver for the current extinction crisis, with the impacts of invasive species expected to be proportional to the relative densities of the invasive species (Ricciardi, 2003; Muñoz and Cavieres, 2008). For example, Jansen and Jude (2001) reported that the productivity failure and eventual extirpation of the Mottled sculpin (*Cottus bairii*) within Lake Michigan was directly correlated with increasing density of the invasive Round goby (*Neogobius melanostomus*). Muñoz and Cavieres (2008) reported that the impacts of invasive *Taraxacum officinale* on two endemic species; *Hypochaeris thrincioide* and *Perezia carthamoides* only occurred when *T. officinale* reached high densities. In some systems, high impacts by invasive species can be acute even when invasive species are at relatively lower densities (Benkwitt, 2013). For example, increasing density of lionfish was shown to have only small additive impact on endemic species, whereas a solitary lionfish had the largest effect on local species richness (Benkwitt, 2013).

A common management tool is the reduction of the invasive species density. For example, feral cat and rat eradication is a common method utilized for increasing insular seabird nesting success (Nogales et al., 2004; Hilton and Cuthbert, 2010). By contrast, removal of lionfish had minimal benefits as cleared areas were rapidly recolonized (Dahl et al., 2016). That is why when managing systems, the vagility and colonization capacity of invasive species should be evaluated prior to expensive removal approaches (Dahl et al., 2016).

Crayfish, as another invasive species are known to be highly vagile and threaten the recovery of numerous desert fish species (Williams, 1985; Guan, 1997, 1998; Rogowski and Stockwell 2006; Thomas and Taylor, 2013). Furthermore, crayfish are capable of rapid population growth, with a single female laying up to 400 eggs biannually (Oluoch, 1990).

Crayfish in high densities have been shown to significantly impact aquatic vegetation, macroinvertebrates, and small benthic fishes (Creed, 2004; Dorn and Wojdak, 2004). Impacts of crayfish on small benthic fishes may be positively correlated with crayfish densities (Rogowski and Stockwell 2006), but a recent study suggested that impacts were highest when crayfish were at low densities (Thomas and Taylor 2013). In the latter study, limited impacts by crayfish at high densities were attributed to interference competition (Thomas and Taylor, 2013). Such interference can increase prey handling time, allowing prey to escape (Case and Gilpin, 1974). Both Thomas and Taylor (2013) and Rogowski and Stockwell (2006) used similar mesocosm approaches to determine the ecological impacts of crayfish on benthic fishes, and yet found two distinct outcomes. This is significant, as crayfish have invaded many sensitive regions, such as aquatic habitats of the Southwestern United States. Fishes within these habitats typically evolved in single species ecosystems, and thus are theorized to be naïve to predation risk posed by invasive species (Miller 1961; Minckley and Deacon 1968; Courtenay and Deacon 1983; Cox and Lima 2006). Invasive species can have acute impacts on numerous desert fishes, making it important to understand if the impact of the invasive species is density dependent. Information on density-dependent effects of crayfish may be useful for evaluating various management strategies such as targeted harvest of crayfish. This is a common management option for many invasive species. Mechanical removal using trapping/netting is both cost-effective and beneficial for removing a large percentage of the invasive species (Holdich et al., 1999; Gaeta et al., 2015).

In the current study, we examine if impacts on endemic species are dependent on crayfish density, and if limiting intraspecific interactions among crayfish mitigate the outcome within high-density treatments found by Thomas and Taylor (2013). We used a mesocosm design to test the effects of red swamp crayfish on experimental populations of Amargosa pupfish (*Cyprinodon* *nevadensis amargosae*). We tested crayfish impacts by evaluating pupfish juvenile productivity and adult survivorship among; I) allopatric pupfish populations. II) pupfish populations in the presence of crayfish at low density, III) pupfish sympatric with crayfish at high densities, and IV) pupfish sympatric with tethered high-density crayfish. The fourth treatment allowed us to evaluate the concept of intraspecific interactions influencing crayfish impacts on pupfish juvenile production and adult survivorship.

4.3. Methods

Experimental communities were established in circular 1211-liter rigid plastic tanks on North Dakota State University's Agricultural Experiment Station in Fargo, ND. Thirty-two mesocosms were set up with approximately 950-l of dechlorinated water, with water levels maintained through additions of dechlorinated water. Each tank contained one cubic foot of river rock, artificial cover material (Five 0.5m-long clumps of plastic mesh weighted to simulate aquatic plants) added uniformly in all tanks to create breeding substrate, and three reclaimed PVC vinyl structures Fishiding® simulating aquatic vegetation. The mesocosms were inoculated with a mixture of plankton from a local semi-permanent wetland three weeks prior to fish additions. All tanks were aerated with an air stone to maintain dissolved oxygen of around 8-9mg/L. The water temperatures changed relative to environmental conditions in a diel rhythm and over the course of both experiments. Tanks were checked daily for mortalities and to assure continuous airflow. Regular water quality tests revealed non-stressful conditions for dissolved oxygen, ammonia, and nitrate levels.

Amargosa pupfish were collected from River Springs in Mono County, CA, while red swamp crayfish were sourced from Carolina Biological suppliers. We used a randomized block design with eight blocks of the following four treatments: I.) allopatric pupfish, II.) sympatric pupfish with low-density crayfish, III.) sympatric pupfish with high-density crayfish, and IV.) sympatric pupfish with high-density tethered crayfish. The final treatment of high-density tethered crayfish was compared only to the high-density crayfish to test if limiting interference competition influenced pupfish survival. All tanks received 8 pupfish with an approximate sex ratio of three females: one male. Low and high crayfish densities were one and four crayfish per tank respectively. Crayfish were not sexed within the experiment, as there was limited availability of overall crayfish. Deceased crayfish were replaced with crayfish of similar size.

For the tethered-crayfish treatment, crayfish were tethered by attaching a small loop of fishing line to a 0.1mm flexible metal wire, with the fishing line being glued to the carapace and held in place by a small .25mm X .25mm square of parafilm (Figure 4.1a). Fishing line was utilized, as the wire itself would not adhere to the carapace of a crayfish. The lines were anchored to the edge of the tank every 90° to limit interactions among crayfish (Figure 4.1b). Crayfish were checked daily to ensure that tethers were intact and to re-tether as necessary to minimize conspecific interactions.

Supplemental tropical fish food was provided every day within each tank at rates of ~2-3% of total fish biomass. We also provided Aquatic Arts sinking pellets as a supplemental food source for crayfish twice weekly at rates ~5% of the overall crayfish biomass.

The experiment was terminated after eight weeks, and we enumerated the number of adult and juvenile pupfish for each tank. All data collected were analyzed using JMP Pro 14 software. Non-parametric Wilcoxon comparison of means were conducted to analyze juvenile productivity, while using block as a factor. This analysis was followed by post-hoc Wilcoxon Each-Pair Comparisons while maintaining an experiment-wise alpha level of 0.05.

Figure 4.1. Image of a tethered crayfish.

Figure 4.2. Image of mesocosm design, demonstrating the 90° tethering, with red circles outlining tethers.

4.4. Results

The mesocosms appeared to provide adequate environmental conditions, as allopatric pupfish communities had overall high adult survivorship and high juvenile productivity. The presence of crayfish had a significant impact on adult pupfish survival ($x^2 = 10.59, p = 0.005$). When allopatric, adult pupfish survival was 96.9% ± 0.03 (mean percent survival ± standard error), significantly higher than pupfish adult survival when sympatric with crayfish both at low density (84.4% ± 0.05; z=-2.15; p=0.03) and at high density (Figure 4.3)(68.8% ±.06; z=-2.84; p=0.0045). Adult pupfish survivorship was similar for populations sympatric with crayfish at low density compared to populations sympatric with high-density crayfish (z=1.77; p=0.077; Figure 4.3).

The presence of crayfish had a significant impact on the number of pupfish juveniles produced ($x^2 = 15.81, p = 0.0004$). Juvenile production did not differ between the allopatric (704 + 77 juveniles per mesocosm) and low-density crayfish (705±33) treatments (z=-.94; p=0.34), but both of these treatments had significantly higher juvenile production than the high-density crayfish treatment (271 ±36 z=3.31; p=0.0009; and z=-3.31; p=0.0009, respectively; Figure 4.4).

For the two high-density crayfish treatments, tethering crayfish had no significant effect on pupfish adult survivorship (70.3%±.05; z=.22; p=.82; Figure 4.5). For the two high-density crayfish treatments, tethering caused a significant increase in juvenile production (493±94) compared to the high density free-ranging crayfish (271±36) (z=2.257; p=.026; Figure 4.6).

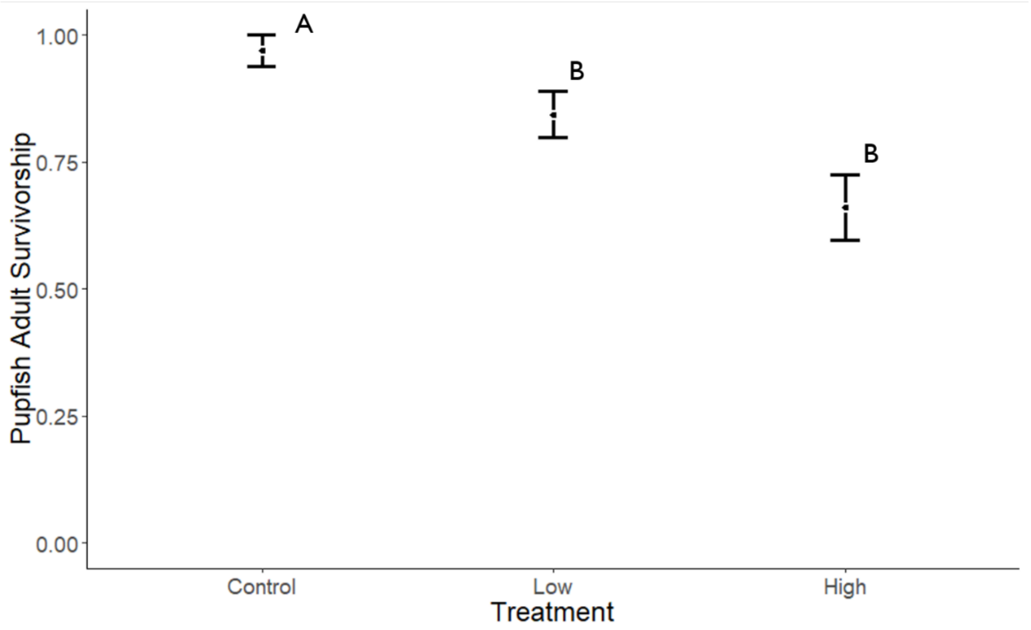

Figure 4.3. Adult pupfish survivorship is shown for pupfish populations averaged across eight replicates within I) allopatry, II) sympatry with low-density crayfish and III) sympatry with high density crayfish. Treatments sharing a letter were not significantly different.

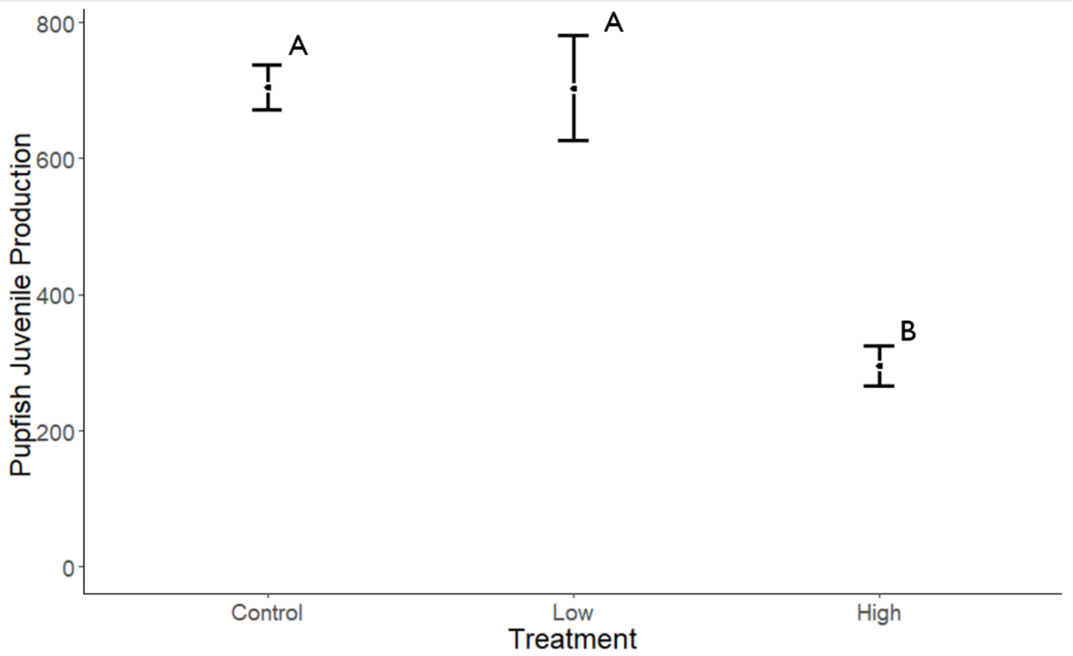

Figure 4.4. Average juvenile productivity from eight replicates are shown for pupfish populations in I.) allopatry, II.) sympatry with low-density crayfish and III.) sympatric with high-density crayfish. Treatments sharing a letter were not significantly different.

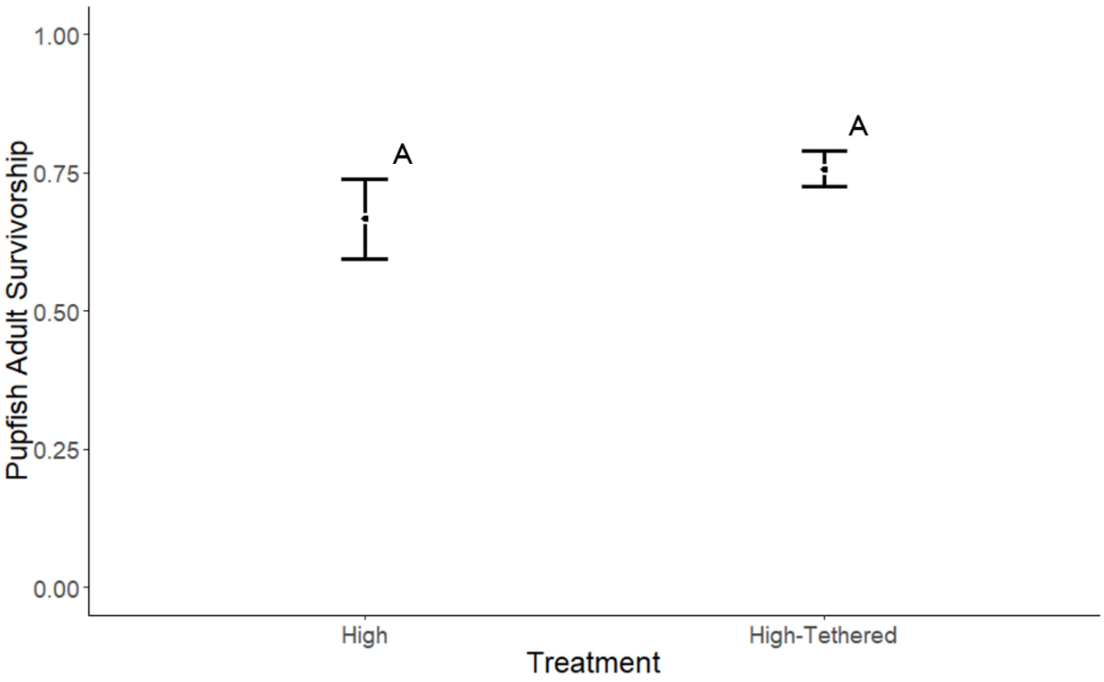

Figure 4.5. Adult pupfish survivorship is shown for pupfish populations averaged across eight replicates within I.) sympatry with high-density crayfish and II.) sympatric with high-density tethered crayfish. Treatments sharing a letter were not significantly different.

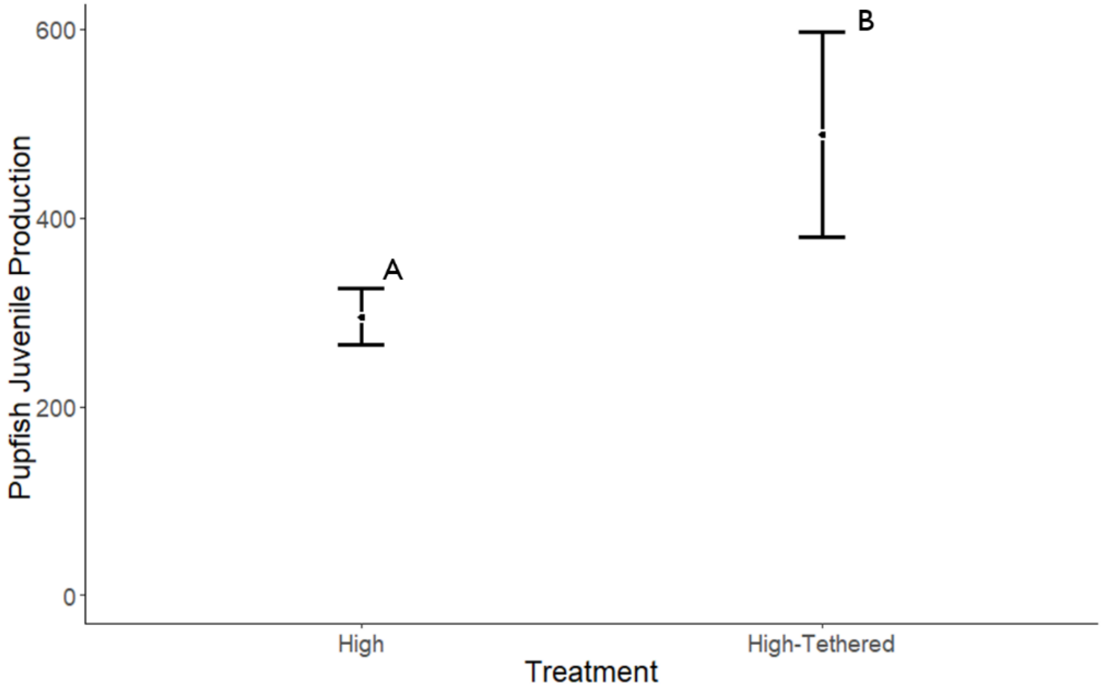

Figure 4.6. Average juvenile productivity from eight replicates are shown for pupfish populations in I.) sympatry with high-density crayfish and II.) sympatric with high-density tethered crayfish. Treatments sharing a letter were not significantly different.

4.5. Discussion

The impacts of invasive species on native species are often proportional to the relative densities of the invasive species (Ricciardi, 2003; Muñoz and Cavieres, 2008). Our results demonstrated that the impacts of crayfish density on Amargosa pupfish adult survivorship corresponded with density of the invasive species. We found significant declines of adult pupfish survival when sympatric with crayfish at both low and high densities. However, adult pupfish survival was noticeably higher in the presence of crayfish at low densities compared to the high-density treatment. This suggests that the mere presence of crayfish may have impacts on overall annual adult survivorship, with increasing density of crayfish having higher likelihood of greater impact.

The effects of crayfish on pupfish juvenile productivity appeared to be associated with increasing crayfish density within our mesocosm experiment. The presence of crayfish in low density had no effect on juvenile pupfish production, however juvenile production declined by over 60% in the presence of crayfish at high density. These results demonstrate reproducible effects similar to earlier work where crayfish impacts on both adult survivorship and juvenile production of White Sands pupfish were density dependent (Rogowski and Stockwell 2006).

This study demonstrated that pupfish populations are likely to decline in response to increasing invasive species density. Similarly, invasive *T. officinale* decreased pollinator visitation rates within *Hypochaeris thrincioide* and *Perezia carthamoides,* but only when the invasive plants were in simulated high population densities of 5 invasive individuals within 30cm radius of a native plant (Muñoz and Cavieres, 2008).

Our results were inconsistent with findings reported by Thomas and Taylor (2013) who reported that crayfish impacts were inversely correlated with crayfish density. They hypothesized that interference competition occurred when crayfish were at high densities, which in turn reduced impacts on benthic fish populations. We used tethering to explicitly test this hypothesis and found higher juvenile production when conspecific interactions were constrained via tethering. We also found that tethering had no effect on pupfish adult survivorship. While tethering may have limited interference competition, it also may have limited crayfish movement which in turn limited impacts on pupfish survival.

We recognize a limitation of our experimental design. We had used commercially available crayfish as opposed to wild caught red swamp crayfish as used in earlier studies (Rogowski and Stockwell 2006; Thomas and Taylor 2013). It has been shown that intraspecific aggression within crayfish may differ between the species level, thus effects on certain benthic fishes may be species specific regarding the invasive crayfish (Atkinson 1989; Blumstein and Daniel, 2005; Pintor et al., 2008). Additional research project utilizing other crayfish species, such as the Virile crayfish and Signal crayfish as used in Rogowski (2006) and Thomas and Taylor (2013), respectively. The usage of different crayfish species may be useful for evaluating if naturally sourced red swamp crayfish have different effects compared to crayfish sourced from biological suppliers, as well as species specific variation amongst effects on benthic fishes. The difference in source populations may have differences in conspecific interactions, however, would likely still result in density dependent effects on pupfish populations (Rogowski and Stockwell, 2006).

Our results suggest that reducing crayfish densities may be an effective management tool to minimize crayfish impacts on pupfish populations. However, continual harvest is probably necessary because female crayfish are capable of laying ~400 eggs within one spawning event, resulting in revitalization of a population if overharvest does not provide a complete removal

(Holdich et al., 1999). In fact, such active management has been applied for controlling invasive crayfish and western mosquitofish populations within Ash Meadows National Wildlife Refuge (Scoppettone et al., 2011). The mechanical removal consists of actively placing pyramid traps, where once a crayfish has entered the trap, they are no longer capable of leaving, without the possibility of trapping small fishes. Annual mechanical removal of crayfish from Bradford-1 spring has demonstrated positive effects compared to population estimates for previous years without removal for both Speckled Dace (*Rhinichthys osculus*) and Ash Meadows Amargosa pupfish densities. This suggests that controlling density of crayfish may help to mitigate negative impacts on endemic populations (Scoppettone et al., 2011). Our results combined with these observations suggest that a similar approach may be profitable for the conservation of desert fishes within this region.

4.6. References

Atkinson, I. 1989. Introduced animals and extinctions. Conservation for the Twenty-First Century. Editors D. Western, M. Pearl 59-75.

Benkwitt, C. E. 2013. Density-dependent growth in invasive lionfish (*Pterois volitans*). PLoS One 8:e66995.

Blumstein, D. T., and J. C. Daniel. 2005. The loss of anti-predator behavior following isolation on islands. Proceedings of the Royal Society B: Biological Sciences 272:1663-1668.

Case, T. J., and M. E. Gilpin. 1974. Interference competition and niche theory. Proceedings of the National Academy of Sciences 71:3073-3077.

Clavero, M., and E. García-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution 20:110.

Courtenay Jr, W. R., and J. E. Deacon. 1983. Fish introductions in the American southwest: A case history of Rogers spring, Nevada. The Southwestern Naturalist 1983:221-224.

Cox, J. G., and S. L. Lima. 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology and Evolution 21:674-680.

Creed Jr, R. P., and J. M. Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. Journal of the North American Benthological Society 23:224-236.

Dahl, K. A., W. F. Patterson III, and R. A. Snyder. 2016. Experimental assessment of lionfish removals to mitigate reef fish community shifts on northern Gulf of Mexico artificial reefs. Marine Ecology Progress Series 558:207-221.

Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. Oecologia 140:150-159.

Gaeta, J. W., T. R. Hrabik, G. G. Sass, B. M. Roth, S. J. Gilbert, and M. J. Vander Zanden. 2015. A whole-lake experiment to control invasive rainbow smelt (Actinoperygii osmeridae) via overharvest and a food web manipulation. Hydrobiologia 746:433-444.

Guan, R., and P. R. Wiles. 1997. Ecological impact of introduced crayfish on benthic fishes in a British lowland river. Conservation Biology 11:641-647.

Guan, R., and P. R. Wiles. 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. Aquaculture 169:177-193.

Hilton, G. M., and R. J. Cuthbert. 2010. The catastrophic impact of invasive mammalian predators on birds of the UK overseas territories: A review and synthesis. Ibis 152:443-458.

Holdich, D. M., R. Gydemo, and W. D. Rogers. 1999. A review of possible methods for controlling nuisance populations of alien crayfish. Crayfish in Europe as Alien Species. How to make the Best of a Bad Situation 1999:245-270.

Janssen, J., and D. J. Jude. 2001. Productivity failure of mottled sculpin *Cottus bairdi* in calumet harbor, southern lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. Journal of Great Lakes Research 27:319-328.

Jude, D. J., R. H. Reider, and G. R. Smith. 1992. Establishment of Gobiidae in the Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 49:416-421.

Miller, R. R. 1961. Man and the changing fish fauna of the American southwest. Michigan Academy of Science, Arts, and Letters, 1961.

Minckley, W. L., and J. E. Deacon. 1968. Southwestern fishes and the enigma of" endangered species". Science 159:1424-1432.

Muñoz, A. A., and L. A. Cavieres. 2008. The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. Journal of Ecology 96:459-467.

Nogales, M., A. Martín, B. R. Tershy, C. J. Donlan, D. Veitch, N. Puerta, B. Wood, and J. Alonso. 2004. A review of feral cat eradication on islands. Conservation Biology 18:310-319.

Oluoch, A. O. 1990. Breeding biology of the Louisiana red swamp crayfish Procambarus clarkii girard in lake naivasha, Kenya. Hydrobiologia 208:85-92.

Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos 117:1629-1636.

Ricciardi, A. 2003. Predicting the impacts of an introduced species from its invasion history: An empirical approach applied to zebra mussel invasions. Freshwater Biology 48:972-981.

Rogowski, D. L., and C. A. Stockwell. 2006. Assessment of potential impacts of exotic species on populations of a threatened species, white sands pupfish, Cyprinodon tularosa. Biological Invasions 8:79-87.

Scoppettone, G. G., P. H. Rissler, C. Gourley, and C. Martinez. 2005. Habitat restoration as a means of controlling non-native fish in a Mojave Desert oasis. Restoration Ecology 13:247-256.

Scoppettone, G.G., Rissler, Peter, Johnson, Danielle, and Hereford, Mark, 2011, Relative abundance and distribution of fishes and crayfish at Ash Meadows National Wildlife Refuge, Nye County, Nevada, 2007–08: U.S. Geological Survey Open-File Report 2011-1017, 56 p.

Thomas, C. L., and C. A. Taylor. 2013. Scavenger or predator? examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. Freshwater Science 32: 1309-1317.

Williams, J. E., and D. W. Sada. 1985. Status of two endangered fishes, Cyprinodon nevadensis mionectes and Rhinichthys osculus nevadensis, from two springs in ash meadows, Nevada. The Southwestern Naturalist 30:475-484.