LIFE HISTORY AND INTERSPECIFIC CO-PERSISTENCE OF NATIVE IMPERILED FISHES IN SINGLE SPECIES AND MULTI-SPECIES *EX SITU* REFUGES

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Shawn Christopher Goodchild

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Program:
Environmental and Conservation Sciences
Option: Conservation Biology

January 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title

LIFE HISTORY AND INTERSPECIFIC CO-PERSISTENCE OF NATIVE IMPERILED FISHES IN SINGLE SPECIES AND MULTI-SPECIES *EX SITU* REFUGES

By

Shawn Christopher Goodchild

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Craig Stockwell
Chair

Dr. Mark Clark

Dr. Donna Jacobs

Dr. Steven Travers

Dr. Marion Harris

Approved:

April 5, 2016
Date

Dr. Eakalak Khan
Department Chair

ABSTRACT

Like many imperiled fishes, the endangered Pahrump Poolfish (*Empetrichthys latos latos*) is managed in *ex situ* refuges. I investigated life history characteristics of females from two such populations at Lake Harriet and Shoshone Stock Pond. Lake Harriet is a relatively large lake with low fish densities located at relatively low elevation and low latitude, while Shoshone Stock is a small pond with high fish densities at a higher elevation and latitude. Females from the Lake Harriet population were larger, and had greater fat content, reproductive allocation, and 'clutch' size than females from the Shoshone Pond population. This divergence, which occurred in three decades, may result in a phenotypic mismatch if the fish are used as a source for restocking their native habitat or stocking new refuges.

Poolfish conservation may require establishing new populations; however, many sites are inhabited by non-native fish and/or other protected fish species. Thus, managers may wish to consider establishing multi-species refuges that may even already include undesirable species. I established experimental communities that included allopatric and sympatric communities of Poolfish, Amargosa Pupfish (*Cyprinodon nevadensis*), and invasive Western Mosquitofish (*Gambusia affinis*). Pupfish persisted in sympatry with both poolfish and mosquitofish, but had higher juvenile production when maintained in allopatry. By contrast, poolfish juvenile production was high in allopatry, but virtually absent in the presence of other species.

To evaluate the generality of these findings, I established experimental allopatric and sympatric communities of poolfish or pupfish with mosquitofish from two populations that differed in body size: Garrett mosquitofish were approximately twice the mass of Wabuska mosquitofish. Poolfish juveniles had high survival in allopatry, but produced virtually no juveniles when sympatric with either of the two mosquitofish populations. Pupfish juvenile survival was higher in allopatry than sympatric with Garrett mosquitofish, which in turn was higher than sympatric with Wabuska mosquitofish. These results were consistent with the earlier experiment suggesting that poolfish were functionally extirpated but pupfish maintained substantial production in the presence of mosquitofish. These findings suggest that poolfish should be maintained in single species refuges, but that multi-species refuges may protect imperiled pupfish species.

ACKNOWLEDGEMENTS

This dissertation was built with the support of many people to whom I am deeply grateful. I am blessed to have so many wonderful friends and family that stood behind me the entire process. The Nevada Department of Wildlife provided funding and assistance with this project. Jon Sjöberg, who has always been a role model for me, was integral to the funding and emotional support. Kevin Guadalupe provided critical field assistance, valuable discussions, and all-around support, and I feel the aquatic critters of southern Nevada are in his safe hands. I also thank Heath Korell and Brandon Senger for their assistance. The United States Fish and Wildlife Service, primarily Lee Simons and James Harter, provided administrative and field assistance. Lee Simons went above and beyond to support this project and myself. Lee is truly an inspiration. Rick Keller of the Nevada Division of State Parks provided support for work at Spring Mountain Ranch, and Nancy Herms of the Bureau of Land Management, Ely, supported work at Shoshone Ponds. Hal Fairfield, Heather Hundt, Allison Manwaring, and Makenzie Stockwell assisted with field work. Hal has done impressive work with the husbandry of Poolfish from which I learned a great deal, and I am honored to have collaborated with him. North Dakota State students Natasha Westen, Samantha Skinner, Samantha Kainz, and Amanda Sopato provided assistance with the grunt work, processing field samples and experimental work.

I am very grateful for having Dr. Craig Stockwell as my advisor and mentor, who brought me into his lab and molded me into a scientist. He also spent a great deal of time in the field collecting and shipping fish for the experiments, and put up with my many deficiencies. Members of Dr. Stockwell's lab also provided invaluable guidance and assistance, including Sujan Henkanaththegedara, Justin Fisher, Kevin Purcell, Vanessa Aparicio, and Kyle McLean. I thank Dr. David Wittrock, Dean of the NDSU College of Graduate and Interdisciplinary Studies, as well as Dr. Adnan Akyuz and Dr. Ken Grafton of the North Dakota Agriculture Experiment Station. I also thank Dr. Eakalak Khan, Program Director of the Environmental and Conservations Sciences Graduate Program, and Dr. Wendy Reed, Associate Professor and Head of NDSU Department of Biological Sciences, for their support of my education. I would also like to thank my committee members: Drs. Mark Clark, Steven Travers, Donna Jacob, and Marion Harris for their guidance, encouragement, and constructive critique of my work.

Lastly, I want to thank my family. My wife, Heather Hundt, kept me on my feet and was the primary reason for my success. My parents (Dennis and Linda Goodchild) and grandparents (Edward and Helen Goodchild, Olin and Rosie Whitaker) pushed me and were behind me every step. My family, cousins, aunts, uncles, Stan and Sue Hundt, and extended family of good friends, provided a great deal of support. I love you all and I am truly blessed.

DEDICATION

This dissertation is dedicated to:

Dr. Jim Deacon, who mentored me,

the Desert Fishes Council, its past and present members,

and Dr. Stan Harris, who believed in me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance (Statistical).
ANCOVA	Analysis of Covariance (Statistical).
CFS	Cubic Feet per Second (Flow Measurement)
IACUC	Institutional Animal Care and Use Committee.
LSD	Fisher’s Least Significant Difference (Statistical).
MS-222	Tricaine Methanesulfonate (Chemical).
SE	Standard Error (Statistical).

CHAPTER 1. INTRODUCTION

1.1. Conservation, Desert Fishes and Poolfish

Biodiversity is declining globally, and extinctions due to anthropogenic causes are increasing (Pimm et al 1995; Pimm and Raven 2000) as natural resources are progressively stressed due to human population growth and climate change. These impacts have been particularly important for North American freshwater fauna, which has an extinction rate five times more than other vertebrates with at least 123 species extinct since 1900 (Ricciardi and Rasmussen 1999). The risk of extinction is especially problematic within aquatic systems in the Southwestern deserts of the United States (Minckley and Deacon 1969; Deacon and Minckley 1991), where the synergy of non-native species, habitat destruction or modification, and climate change impose anthropogenic challenges for the survival of aquatic species, especially short-lived fish (Hendrickson and Brooks 1991). In fact, many so-called *desert fishes* have been driven to extinction within the last century (Miller et al. 1989), and many other taxa have been regionally extirpated (i.e. Colorado Pikeminnow (*Ptychocheilus lucius*)(Minckley and Deacon 1969). As a result, most desert fishes are legally designated as threatened or endangered in the United States (Fagan et al. 2002) as well as in Mexico (Norma Oficial Mexicana 2002).

The plight of native fishes have led managers to use aggressive conservation measures such as the establishment of refuge populations as a hedge against extinction (refugia per Miller 1969) (Minckley 1995). Such managed relocation has been debated for narrowly endemic aquatic species (Olden et al 2011); however, there is risk in this approach (Storfer 1999; Ricciardi and Simberloff 2009; Olden et al 2011), partially through changes in phenotype or un-anticipated interactions with other taxa inhabiting designated refuge habitats

(Henkanaththegedara and Stockwell 2014). Unintended rapid evolutionary phenotypic divergence of the translocated population has been demonstrated in *ex situ* populations (Reznick and Ghalambor 2001; Stockwell et al 2003; Wilcox and Martin 2006; Collyer et al 2011). Such divergence begs the question of whether these populations are suitable as potential source stock for reintroduction to the original habitat (Stockwell and Weeks 1999; Wilcox and Martin 2006; Collyer et al 2007, 2011).

If refuge populations genetically or phenotypically 'change', then managers may need to adapt management goals and/or methods. One approach would be to allow refuge populations to diverge, addressing reintroduction issues when necessary (Stockwell et al. 2003; Collyer et al. 2011). These refuges would function as a novel ecosystem (per Hobbs et al 2009), and may be beneficial to provide resilient stock in light of large-scale habitat changes predicted from climate change. Another approach is to manage a population that is compatible with reintroduction by changing the physical or ecological characteristics of the fish (Watters et al 2003) (e.g. genetic variation and/or phenotypic variation) and/or the refuge (e.g. biotic and/or abiotic factors) (e.g. Gumm et al 2008). To affect this "change", it is critical to understand how environmental conditions in the refuge affect phenotypes and to identify pertinent selective pressures (Storfer 1999; Lankau et al 2011).

Another threat to both native and *ex situ* refuge populations is the presence of non-native species that may threaten the targeted species of concern. In general, non-native species have been considered detrimental to the persistence of imperiled desert fish (e.g. Meffe 1985; Marsh and Langhorst 1988). The vulnerability of desert fishes to invasive species has been attributed to evolutionary naiveté associated with the relatively species-poor desert systems (Miller 1961; Cox and Lima 2006). This perception has led managers to seek potential refuge sites that are free of invasive species, which has made it even more challenging to find suitable refuge habitats. However, recent work has shown that the impacts of invasive Western mosquitofish (*Gambusia affinis*) on protected Mohave tui chub (*Gila bicolor mohavensis*) may be mediated by intra-guild predation, whereby adult tui chub consume adult mosquitofish, while adult mosquitofish prey on tui chub larvae (Henkanaththegedara and Stockwell 2012, 2014).

These findings suggest that differences in key life history traits among populations and/or species may mediate the effects of non-native on native species. Consequently, caution must be used when interpreting literature on determining effects of introduced species, since effects of introduced species may be context specific on various abiotic and biotic factors (i.e. Dunson and Travis 1991). Further, the impacts of invasive predators may be mediated by gape size limitations (Henkanaththegedara and Stockwell 2012, 2014; Hambright 1991; Nilsson and Bronmark 2000; Magnhagen and Heibo 2001; Webb and Shine 1993). Therefore, gape-size and associated predator size, along with site-specific population phenotypes of introduced fish and habitat characteristics, may have differing effects when the species exist in sympatry.

Thus, understanding how widespread invasive species interact with native species is critical for conservation planning; for instance, potential refuge sites often contain alien species, such as western mosquitofish (*Gambusia affinis*). The presence of mosquitofish is particularly noteworthy because this species is listed as a threat to the recovery of many endangered fish species (Henkanaththegedara and Stockwell 2014). However, recent work has suggested that in some cases, native species of concern may be able to co-occur with invasive species such as western mosquitofish (Hekanaththegedara and Stockwell 2012, 2014). These findings challenge pre-conceived notions that refuge populations of endangered species be managed solely in single species refuges. For instance, an important refuge habitat for the endangered Mohave tui chub

(*Gila bicolor mohavensis*) also is occupied by populations of the Saratoga Springs pupfish (*Cyprinodon nevadensis nevadensis*) and the Western mosquitofish (*G. affinis*) (Henkanaththegedara and Stockwell 2014). Thus, novel management scenarios, such as establishing multi-species refuges that even include nuisance species may untie the hands of managers as they seek to recover the numerous endangered desert fish species. However, currently there are no data available to evaluate the benefits and risks of such a novel management approach. A number of factors may facilitate co-persistence among species such as differences in life history, body size and niche breadth.

Given these complexities it is important to conduct experimental work to investigate the persistence of species of concern when maintained in "multi-species refuges". Such work should also further evaluate the universal impacts of non-native species. From these results, managers may adapt obsolete refuge management to better suit a changing 21st Century world.

The complexities of aquatic species conservation are well illustrated by the poolfishes (*Empetrichthys* spp.), which historically occupied a variety of habitats in southwestern Nevada. Poolfish along with springfish (*Crenichthys sp.*) are in the subfamily Empetrichthyinae and are the only egg-laying fishes within the Goodeidae family (Parenti 1981). One of the two species of *Empetrichthys*, the Ash Meadows Killifish (*E. merriami*) was extinct by the 1950's, and its extinction coincided with habitat alterations and the introduction of non-native species (Miller et al 1989). The other species, *E. latos* included three subspecies, each occurring in its own spring system within the Pahrump Valley, Nye County, Nevada. The Raycraft Spring poolfish (*E. latos concavus*) and the Pahrump Ranch killifish (*E. latos pahrump*) both became extinct when their respective habitats were destroyed in the 1950's (Miller et al 1989). Thus, the Pahrump poolfish

(*Empetrichthys latos latos*) that historically occupied Manse Spring is the only extant taxon within *Empetrichthys*.

Like many desert fishes, the Pahrump Poolfish persisted in a presumably species-poor system for between 10 and 20 thousand years (Soltz and Naiman 1978), but poolfish have maintained populations in an Euro-American-altered environment for at least 130 years (see McCraken (2009) for the history of Manse Ranch, which includes Manse Spring). Population estimates of the native poolfish population at Manse Spring were compiled between 1937 and 1975 (Deacon and Williams 2011). During this time, the population varied in size from less than 50 to more than 1000 individuals. Two major population crashes to less than 50 individuals occurred during this time period, which were attributed to vegetation removal and goldfish eradication efforts (Deacon and Williams 2011). The species was preserved by translocating fish to three *ex situ* habitats prior to the spring failure in 1975 (Deacon and Williams 2011) (Figure 1). Two of these transplanted populations ultimately failed, but a population at Corn Creek that was established in August of 1971 with 29 founders become the source stock for all extant populations (Figure 1.1). Subsequently, on August 31, 1976, 66 fish from the Corn Creek refugium were used to establish a population at Shoshone Ponds near Ely, Nevada. Fish have persisted at this site among a number of proximate habitats including a stock pond, a small stream below a wellhead and among two small nearby ponds. On June 9, 1983, 426 fish from Corn Creek were transplanted to Lake Harriet at Spring Mountain State Park, just west of Las Vegas, Nevada, and this population has persisted since that time. In 1988, the Corn Creek population was extirpated which was attributed to the introduction of crayfish (*Procambarus clarkii*) and western mosquitofish. Lake Harriet poolfish were used to establish populations within two above-ground tanks (30 fish each) in 2003. In 2013 the pond at Corn Creek was renovated, and poolfish from Spring Mountain Ranch were re-introduced on August 18, 2014 (n = 175) and on August 21, 2014 (n=500)(Kevin Guadalupe, personal communication). This population was augmented with 500 more poolfish from Lake Harriet on June 16, 2015(Kevin Guadalupe, personal communication).

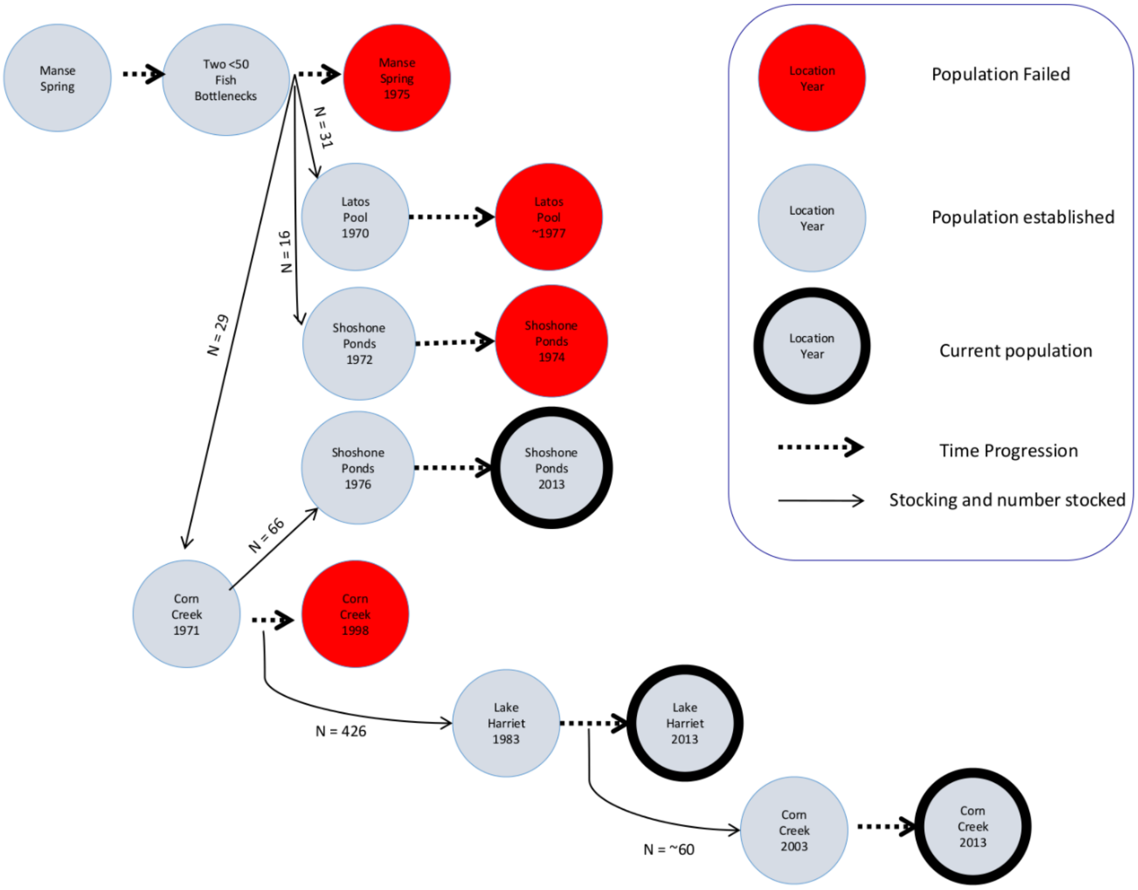

Figure 1.1: Stocking and population history of Pahrump Poolfish (*Empetrichthys latos latos*) to 2013.

The Pahrump Poolfish thus provides a unique study system to investigate phenotypic variation and other ecological conditions among *ex situ* populations, as well as community interactions (e.g. predation). Although the native population has been extirpated in Manse

Spring, five other locations have hosted isolated populations for at least three decades. Translocations typically involved small numbers of individuals with smaller effective population sizes. The historic population contributions and subsequent transplants have likely reduced the genetic variation in this species. As of January 2016, six populations of *E. latos* persist at three geographical locations (Figure 2.1). These refuge populations occupy very different habitats from the original spring, including lower temperature lacustrine and stream habitats, and most notably substantial differences in latitude and elevation among the three habitats (Table 1.1).

Table 1.1: Approximate characteristics of Pahrump Poolfish refuge habitats. Confidence intervals are not included.

Name	Shoshone Stock	Shoshone Stream	Lake Harriet	Corn Creek[1]
Latitude	38°56'22.43" N	38°56'11.26"N	36° 4'5.16"N	36°26'21.95"N
Elevation (m)	1768	1768	1164	2500
Average Air Max Temp July (^{o}C)	30.6 (Ely)	30.6 (Ely)	41.1 (LV)	41.1 (LV)
Average Air Min Temp January (^{o}C)	-12.6 (Ely)	-12.6 (Ely)	0.9 (LV)	0.9 (LV)
Surface Area (m^2)	50	25	14,000	n/a
Est. Average Depth (m)	1	0.2	2	n/a
Max Depth (m)	1.5	0.4	7	n/a
Estimated volume (m^3)	50	5	25,900	n/a
Flow (cfs)	~0.05	~0.1	0.45	n/a
Latest Population	2,919	518	31,570	(49/13)
Inflow Water Temp (^{o}C)	20	24	variable	variable
Outflow Length (m)	pond	51	pond	n/a

Commonalities among locations include the anthropogenic-constructed habitats, lack of invasive species (prior to 2013), and probable limited variation due to multiple bottlenecks. All

---

[1] The U.S. Fish and Wildlife Service maintained an artificial structure with two separate chambers containing two populations of poolfish (n=49 and n = 13). Poolfish were stocked in constructed ponds at Corn Creek in 2014 (Guadalupe pers com) but no population estimates have been performed as of this writing.

timing of stocking is known, as are historic population data and life history information from Manse Spring (Deacon and Williams 2011). Due to the differences of these sites, there may be differences in phenology, physiology, and phenotypes (e.g. size, behavior). Thus, I measured phenotypic variation within and among populations and considered local environmental factors that may drive any possible divergence.

Understanding if populations have undergone phenotypic divergence, is important to develop management plan for all populations. For example, if populations have diverged, gene flow from other sites can compromise local adaptation (Storfer 1999). Thus, one option may be to treat populations as separate management units (sensu Moritz 1994), and allow populations to locally adapt. The security of these populations is still of concern due to ground water pumping and the risk of non-native species introduction, thus conservation of this species is focused on securing the extant populations, and exploring opportunities to establish additional refuge populations. This may be a viable strategy for desert aquatic fauna, especially if the native habitat of a species was destroyed, but it will be a paradigm shift from the original purpose of refuges (Miller 1969).

This dissertation will address life history variation and community ecology of the Pahrump poolfish to provide guidance for management for this species, as well as other imperiled aquatic fauna. I will focus on the two typically largest populations of poolfish, at Shoshone Stock Pond and Lake Harriet. These habitats differ in inflow, elevation, aspect (Shoshone is at the western base of a large mountain chain, and Lake Harriet is at the base of a smaller eastern face), and latitude, setting the stage for possible unique selective regimes. It is useful to note that the three subspecies of E. latos (*E. latos latos, E. l. Pahrump and E. l. concavus*) occupied habitats with similar thermal and other habitat conditions, and yet diversified after isolation at the end of the Pleistocene. If the phenotypic variation of these three subspecies differed in similar habitats, the inter-population variation for the extant refuge populations of *E. latos* may also vary given the drastically different environments.

This dissertation will explore three topics: 1) Life history variation among populations of poolfish within currently established refuges, 2) use of multi-species refuges as a potential tool to streamline management, and 3) effects of mosquitofish with differing population phenotypes on the persistence of poolfish relative to these refuges.

We hypothesize that:

1) There will be life history differences due to the different conditions in the refuge habitats (Chapter 2);

2) poolfish or pupfish may coexist in multi-species communities (Chapter 3); and

3) mosquitofish of differing size phenotypes would have different impacts on poolfish larval survival (Chapter 4).

1.2. Study Sites

Three sites currently host Poolfish populations in Nevada (Figure 1.2). I will briefly describe the habitat characteristics of the two study sites, as well as Manse Spring. I will also summarize the habitats for the extinct subspecies that occurred at Pahrump Ranch Spring and Raycraft Ranch Spring, the extinct sister species *E. merriami*, and two other refuge sites (Table 1.2). Manse Spring, within the Pahrump Valley, Nye County, Nevada, is located on the outskirts of Pahrump, at 850 m elevation. Manse Spring was a warm, alkaline spring that maintained a relatively constant temperature of 24° C (Selby 1977), with daily and annual fluctuations varying from 23.3° to 25.0° C (Miller 1948; Sigler and Sigler 1987).

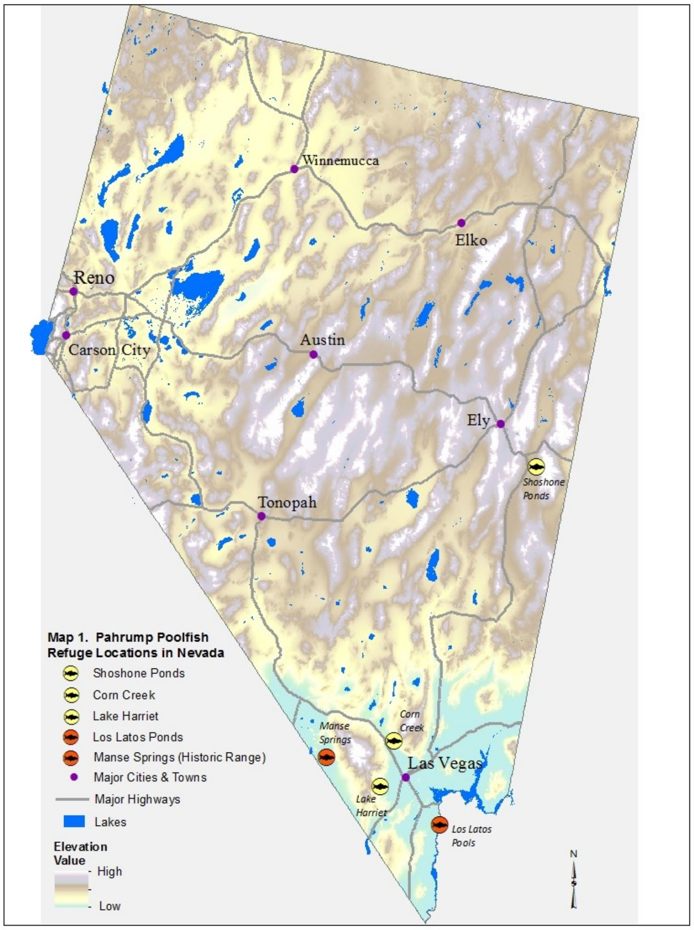

Figure 1.2: Current Pahrump Poolfish refuge locations in Nevada (2013). Red circles indicate historic but extirpated poolfish locations which no longer harbor poolfish, yellow are extant populations.

Miller (1948) described Manse Spring as having a 167 m^2 surface area and between 0.3 to 2 meters deep. It had a silt bottom with watercress (*Nasturtium*), *Chara*, green algae, and a fine-leaved *Potamogeton*. Miller (1948) noted the pool was shaded by cottonwoods (*Populus*); however, photos from about 100 years earlier (circa 1850; McCraken 2009) show ash (*Fraxinus*), willows (*Salix*), and mesquite (*Prosopis*), with an understory of Yerba Mansa

(*Anemopsis californica*) and sedge (*Caryx*). Miller (1948) described the current as "moderate in the pool and swift in the outlet", and the water was clear. The outlet now flows approximately 75m into a constructed series of ponds. Prior to 1970, the springhead was piped (NDOW 1970 unpublished report), suggesting a history of manipulation, and photos from the 1890's (McCraken 2009) show a pool. Outflow temperatures were likely variable, as opposed to the measurements taken in the spring pool.

Table 1.2: Approximate Characteristics of historic Pahrump Poolfish habitats.

Name and Poolfish taxa	*E. merriami* Ash Meadows (Multiple Springs)	*E. l. Pahrump* Pahrump Ranch	*E. l. concavus* Raycraft Ranch	*E. l. latos* Manse Spring
Latitude	36°22'28.59"N	36°12'26"N	36°12'35"N	36° 9'19.47"N
Elevation (m)	1768	2713	2654	853
Average Air Maximum Temp July (°C)	39.4	37.8	37.8	37.8
Average Air Minimum Temp January (°C)	-0.6	-2.2	-2.2	-2.2
Surface Area (m^2)	Variable, 188 Max	45.5	Marsh	167
Est. Average Depth (m)	Variable 4 Max	1.1	Shallow	1
Maximum Depth (m)	Variable, 7 Max	1.2	Small	2
Estimated volume (m^3)	Variable, ~940 Max	50	Small	47
Flow (cfs)	Max 2.3 cfs	4.73	0.022	2.67
Inflow Water Temp (°C)	Variable, Approx. 20	24.7	25.3	24
Outflow Length (m)	Variable, 5 springs Up to 1,800	Unknown	Unknown	75

1.2.1. Shoshone Stock Pond

The Shoshone Stock Pond (Figure 1.3) is a 0.005 ha constructed pond, receiving water (historically approximately 22 liters/minute (BLM 2014)) from Shoshone Well #4. The outlet of the pond is a small overflow pipe, which was measured to discharge approximately 6.8 liters/minute during summer of 2012. The depth of the pond is between 0.5 to 1.5 meters. The inflow temperature was relatively stable, with the pond temperature fluctuating depending on the time of year (Figure 1.4). The substrate is covered by filamentous algae and *Potamogeton*, under which approximately 0.5 meters of organic silt over a compacted rock bottom. Typically, watercress (*Nasturtium*) occurred only near the inflow, and sparse *Juncus* was present along the shore, but overall the vegetation within the saturated soil at this site has been grazed and is short. A cattle exclosure was built in 2013, and cattle allowed access to the pond periodically. The artesian well began to fail in summer of 2014, which resulted in very low water levels. As of 2015, minimal levels of water relied upon a solar pump placed in the well, which discharged approximately 15 liters/minute (BLM 2014).

Figure 1.3: Undated Google Earth photo of Shoshone Stock Pond, White Pine County, Nevada. The inflow is in the extension on the upper right of the pond.

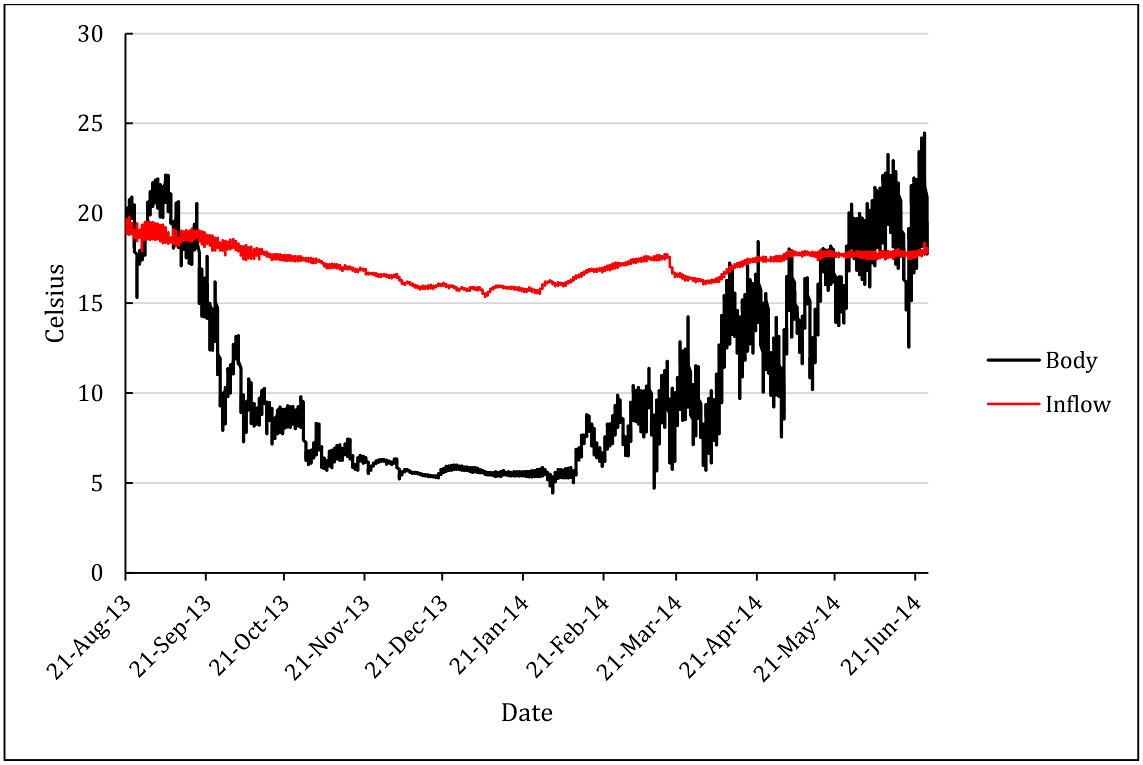

Figure 1.4: Water temperature (2-hour intervals) of inflow and bottom middle of Shoshone Pond.

1.2.2. Lake Harriet – Spring Mountain Ranch

Lake Harriet (Figure 1.5), at 1164m elevation within Spring Mountain Ranch State Park, is a 1.4 ha reservoir receiving flow from a piped spring diversion. Water level in the reservoir is managed using a stopblock structure on a low concrete dam, from which there is a constant discharge due to seepage into an ephemeral flood control basin downstream. Water levels vary regularly due to management needs of the state park, which has included periodical reductions of approximately 25% of its surface area for winter maintenance. Depth at full capacity varies from approximately 0.3m to 7m deep. Water temperature of the inflow was relatively constant, but also changed as managers varied the flow. The lake temperatures were surprisingly consistent at all depths (Figure 1.6), possibly due to circulation from the inflow. Temperatures of the shorelines were variable based on time of day and date (Figure 1.7).

Cattails and small sedges occur in patches around the reservoir edge as well as the edge of a small island, and watercress is present only in the inflow stream. Vegetation, the shoreline, and shallow waters are disturbed by feral donkeys (*Equus asinus*), and there is frequent public visitation of this site. Visible substrate is mainly detritus, flocculent algae, and filamentous algae. Deacon (1985) reported *Chara* (Characea) covering approximately 70% of the rock/sand substrate. Substrate under deeper water is unknown, but during periods of winter drawdown it appeared to be mud, decomposing *Chara*, and dried flocculent material. Invasive non-native crayfish (*Procambarus clarkii*) were first observed in 2013, and continued to increase in abundance (Kevin Guadalupe, NDOW, personal communication).

Figure 1.5: Undated photo of Lake Harriet, Spring Mountain Ranch State Park, Clark County, Nevada. Inflow is at the lower left of the pond, and the outflow is the upper right.

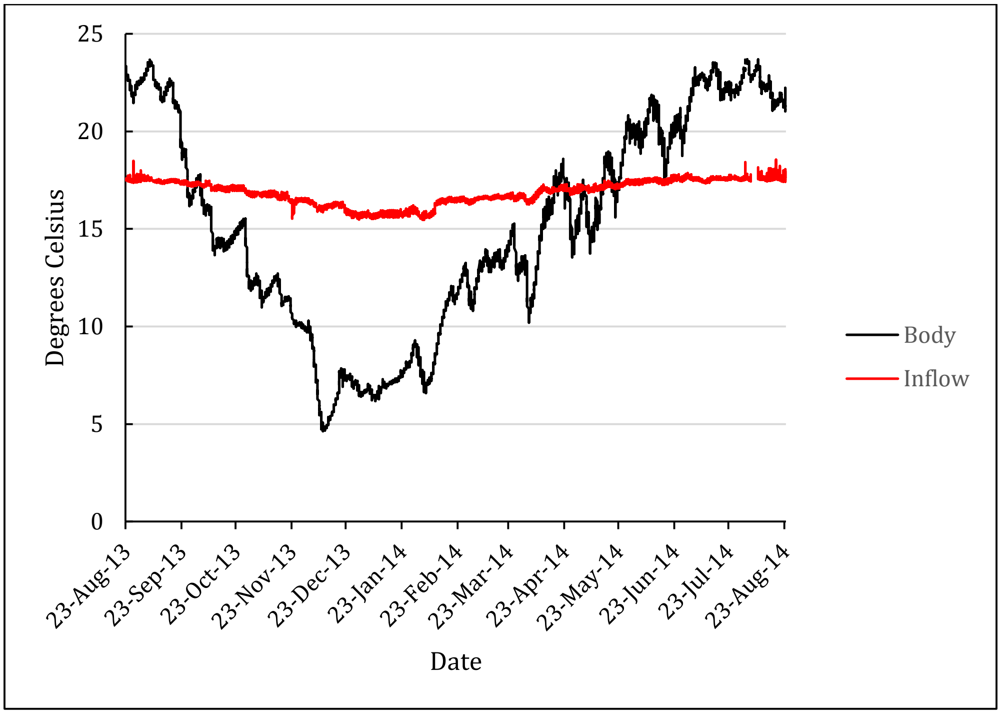

Figure 1.6: Water temperatures (2-hour intervals) of middle water column (body) and inflow of Lake Harriet. Blank in inflow during August 2014 was due to public removing the logger from the inflow.

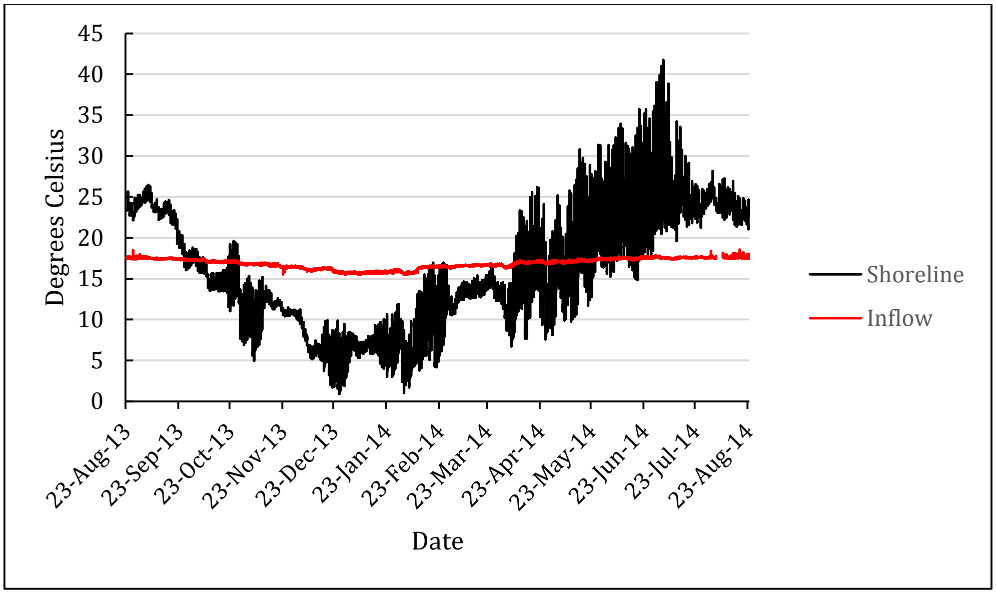

Figure 1.7: Water temperatures (2-hour intervals) of shoreline (inside cattail stand) and inflow of Lake Harriet. Blank in inflow during August 2014 was due to public removing the logger from the inflow.

1.3. References

BLM. 2014. Pahrump Poolfish Habitat Enhancement at the Shoshone Ponds ACEC. SNPLMA Round 15 Conservation Initiative Project Draft Proposal, Bureau of Land Management Ely District, Schell Field Office.

Collyer, M. L., C. A. Stockwell, D. C. Adams, and M. H. Reiser. 2007. Phenotypic plasticity and contemporary evolution in introduced populations: evidence from translocated populations of white sands pupfish (*Cyprinodon tularosa*). Ecological Research 22: 902–910.

Collyer, M. L., J. S. Heilveil, and C. A Stockwell. 2011. Contemporary Evolutionary Divergence for a Protected Species following Assisted Colonization. PLOS ONE 6(8), e22310.

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. 1985. Pahrump Killifish. Endangered Species Information System, Species Workbook. Unpublished Report to the U.S. Fish and Wildlife Service.

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.

Deacon, J. E. and W. L. Minckley. 1991. Western fishes and the real world: the enigma of “endangered species” revisited. Pp. 405-413. *In*: Battle Against Extinction. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

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

Fagan, W. F., P. J. Unmack, C. Burgess, and W. L. Minckley. 2002. Rarity, Fragmentation, and Extinction Risk in Desert Fishes. Ecology 83: 3250-3256.

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

Hambright, K. D., R. W. Drenner, S. R. McComas, and N. G. Hairstone. 1991. Gape-limited piscivores, planktivore size refuges, and the trophic cascade hypothesis. Archiv für Hydrobiologie 121: 389-404.

Hendrickson, D. A., and J. E. Brooks. 1991. Transplanting short-lived fishes in North American deserts: review, assessment, and recommendations. Pp 283-298. *In*: Battle Against Extinction. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

Henkanaththegedara, S. M. and C. A. Stockwell. 2012. 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.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology and Evolution 24: 599-605.

Lankau, R., P. S. Jørgensen, D. J. Harris, and A. Sih. 2011. Incorporating Evolutionary Principles into Environmental Management and Policy. Evolutionary Applications 4: 315-125.

Magnhagen, C. and E. Heibo. 2001. Gape size allometry in pike reflects variation between lakes in prey availability and relative body depth. Functional Ecology 15: 754-762.

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

McCraken, R. D. 2009. Manse Ranch: Gateway to the Future. Nye County Press, Tonopah, NV.

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, Museum of Zoology, University of Michigan 68: 1-155.

Miller, R. R. 1961. Man and the changing fish fauna of the American Southwest. Papers of the Michigan Academy of Science, Arts and Letters 46: 365–404.

Miller, R. R. 1969. Conservation of Fishes of the Death Valley System in California and Nevada. Transactions of the California-Nevada Section, The Wildlife Society: 107-122.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American Fishes During the Past Century. Fisheries 14: 22-38.

Minckley, W. L. 1995. Translocation as a Tool for Conserving Imperiled Fishes: Experiences in Western United States. Biological Conservation 72: 297-309.

Minckley, W. L. and J. E. Deacon. 1969. Southwestern Fishes and the Enigma of "Endangered Species." Science 159: 1424-1432.

Moritz, C. 1994. Defining "Evolutionary Significant Units" for Conservation. Trends in Ecology and Evolution 9: 373-375.

NDOW 1970. Transplant of Empetrichthys Latos. Unpublished Field Report, Dale Lockard, Nevada Department of Fish and Game, June 9, 1970.

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

Norma Oficial Mexicana. 2002. NOM-059-ECOL-2001: Protección ambiental. Especies nativas de México de flora y fauna silvestres. Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio. Lista de especies en riesgo. Diario Oficial de la Federación, 6 March 2002.

Olden, J. D., M. J. Kennard, J. J. Lawler, and N. L. Poff. 2011. Challenges and Opportunities in the Implementing Managed Relocation for Conservation of Freshwater Species. Conservation Biology 25:40-47.

Parenti, L. R. 1981. A Phylogenetic and Biogeographic Analysis of Cyprinodontiform Fishes (Teleostei, Atherinomorpha). Bulletin of the American Museum of Natural History 168: 335-557.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The Future of Biodiversity. Science 269: 347-350.

Pimm, S. L. and P. Raven. 2000. Biodiversity: Extinction by Numbers. Nature 403: 843-845.

Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112: 183-198.

Ricciardi, A. and J. B. Rasmussen. 1999. Extinction Rates of North American Freshwater Fauna. Conservation Biology 13: 1220-1222.

Ricciardi, A. and D. Simberloff. 2009. Assisted colonization is not a viable conservation strategy. Trends in Ecology and Evolution 24: 248–253.

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

Sigler, W. F. and J. W. Sigler. 1987. Fishes of the Great Basin, a natural history. University of Nevada Press, Reno, NV. 273-275 pp.

Soltz, D. L., and R. J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, Science Series 30. 76pp.

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

Stockwell, C. A., A. P. Hendry, and M. T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution. 18: 94–101

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation 87:173–180.

Watters, J. V., S. C. Lema, and G. A. Nevitt. 2003. Phenotype Management: A New Approach to Habitat Restoration. Biological Conservation 112: 435-445.

Webb, J. K., and R. Shine. 1993. Prey-size selection, gape limitation and predator vulnerability in Australian blindsnakes (Typhlopidae). Animal behaviour 45: 1117-1126.

Wilcox, J. L. and A. P. Martin. 2006. The devil's in the details: genetic and phenotypic divergence between artificial and native populations of the endangered pupfish (Cyprinodon diabolis). Animal Conservation 9: 316–321.

CHAPTER 2. LIFE HISTORY VARIATION BETWEEN *EX SITU* POPULATIONS OF THE ENDANGERED PAHRUMP POOLFISH (*EMPETRICHTHYS LATOS LATOS*).[1]

2.1. Abstract

The endangered Pahrump Poolfish (*Empetrichthys latos latos*) was endemic to one small spring in Nevada, but due to the loss of this habitat now only occurs in *ex situ* refuges that vary widely in elevation and latitude. This has provided an opportunity to investigate life history variation in response to a range of habitats. During the peak breeding season, we investigated the life history characteristics of females from two such populations that occupy a relatively large low-elevation lake and a smaller higher-elevation pond, Lake Harriet and Shoshone Stock Pond, respectively. Females from the Lake Harriet population were larger, and had greater fat content, reproductive allocation and clutch size (number of late-stage oocytes in the asynchronous ovary) than females from the Shoshone Pond population. Compared to Shoshone, the Lake Harriet population displayed greater monthly variation in these life history traits throughout the breeding season. This phenotypic divergence has occurred within a few decades and may reflect a plastic or evolutionary response to environmental factors such as temperature and/or population density. As such, more attention is needed to assess if phenotypic divergence of refuge populations of *P. latos* may result in a phenotypic mismatch to prospective additional refuge habitats and/or reintroduction to their native habitat.

---

[1] The material in this chapter was co-authored by Shawn C. Goodchild and Craig A. Stockwell. Shawn C. Goodchild had the primary responsibility for collecting samples in the field as well as processing samples in the laboratory. Shawn C. Goodchild was the primary developer of the conclusions described here within, drafted and revised all previous versions of this chapter. Craig A. Stockwell served as a proofreader and supplied constructive comments for an improved chapter.

2.2. Introduction

The imperilment of desert fishes in the Southwestern United States (Minckley and Deacon 1991) has led managers to use aggressive conservation measures such as the establishment of refuge populations as a hedge against extinction (refugia per Miller 1969; Minckley 1995). One consequence of this management technique is the unintended phenotypic divergence of the translocated population. For example, three refuge populations of the endangered Devils Hole pupfish (*Cyprinodon diabolis*) all diverged phenotypically in body size and behavior from their parental population (Wilcox and Martin 2006). In a similar case, contemporary evolutionary divergence in body shape was observed for an introduced population of White Sands pupfish (Collyer et al. 2005, 2011). In fact, evolutionary divergence is widely reported for fishes introduced to new habitats (Reznick and Ghalambor 2001; Kinnison and Hairston 2007).

Most cases have documented changes for functional morphological traits and life history traits many of which are considered fitness correlates (Roff 1992; Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Stockwell et al. 2003). Such rapid divergence can result in populations that are maladapted to the original native habitat (Reznick et al. 2004). For fishes, phenotypic divergence of newly established populations has been well documented for poeciliids, cyprinodonts and salmonids (Stockwell and Weeks 1999; Reznick and Ghalambor 2001; Stockwell et al. 2003; Hendry et al. 2000). By contrast, little work has been conducted to evaluate life history variation for fishes restricted to isolated springs with limited environmental variation. However, many such species have been actively managed by establishing refuge populations as a hedge against extinction (Hendrickson and Brooks 1991; Pister 1993; Dunham and Minckley 1998).

One species that has been intensively managed is the Pahrump Poolfish (*Empetrichthys latos*), an endangered species with a complicated history involving numerous refuge populations established at various sites in Nevada (Chapter 1). The subspecies *E. latos latos* was endemic to Manse Spring (Nye County, Nevada), but prior to the desiccation of the spring, 29 individuals were translocated and served as source stock for a network of refuge populations (Figure 1; also see Deacon and Williams 2011). This history includes a number of introductions, extirpation and re-colonization, and as of 2014 six populations persist at three sites in Nevada. These refuge populations occupy very different habitats from the original spring, including lower temperature lotic and lentic habitats.

Here, we describe variation of female life history traits within and between the two largest populations that have persisted for over three decades with no documented additional gene flow (Figure 2.1). We hypothesize that there will be life history differences due to the different conditions in the refuge habitats.

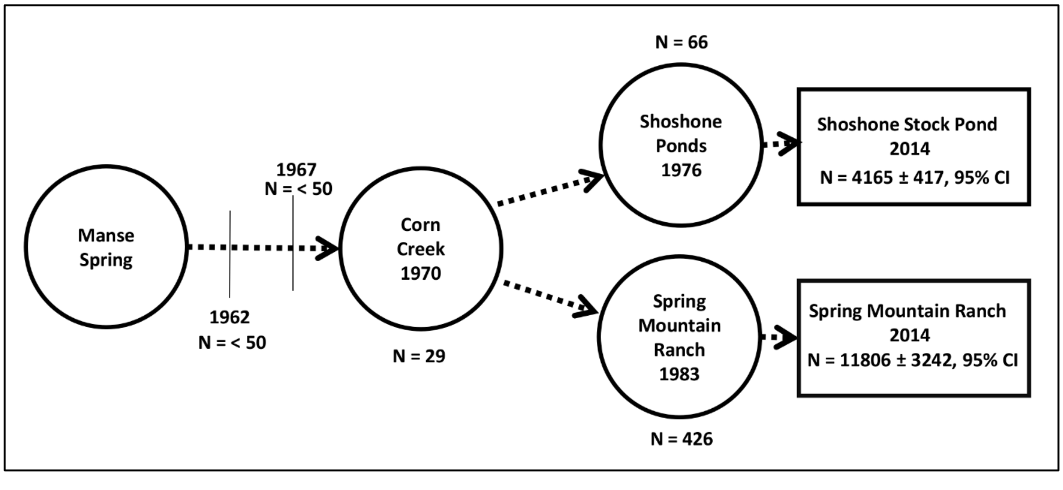

Figure 2.1: History of the Shoshone Stock Pond and Spring Mountain Ranch populations of Pahrump Poolfish. Numbers of fish used to establish populations, dates of notable population declines and stocking events are shown, culminating in a 2014 population estimate (Deacon and Williams, 2011; NDOW, 2014).

2.3. Materials and Methods

2.3.1. Study Sites

Pahrump Poolfish (Poolfish) were collected from the two largest refuge populations: Lake Harriet, Spring Mountain Ranch State Park, Clark County, Nevada, and Shoshone Stock Pond, White Pine County, Nevada (hereafter referred to as the Lake Harriet and Shoshone populations, respectively). The sites were also chosen because they occur at different elevations and latitudes, providing different physical characteristics and likely differences in breeding season duration (Table 2.1).

Table 2.1: Typical characteristics of the study sites and the type locality. Temperature, area, and volume fluctuate given climactic variation, management, location at site, and groundwater conditions; however, these measurements are typical of the differences between the sites during this study (Miller 1948; Deacon and Williams 2011; University of Nevada Desert Research Institute; Kevin Guadalupe (unpublished); Shawn Goodchild, unpublished).

Characteristic	Shoshone	Lake Harriet	Manse Spring
Latitude	38° 56'22.43"N	36° 4' 5.16"N	36° 9' 19.47"N
Elevation (m)	1768	1164	853
Ave Air Maximum Temperature July (°C)	31.3	35.9	38.3
Average Air Minimum Temperature January (°C)	-1.3	-9.7	-1.1
Estimated Volume (m^3)	330	25,900	47
Typical Flow (L/second)	~1.42	2.83	75.6
Estimated 2014 Population Density (number of fish/m^3)	13	0.5	Extirpated
Inflow Water Temp (°C)	20	~15-17	24

The Lake Harriet population was at 1164 m elevation and 36° 4' 5.16"N latitude, and was founded with 426 individuals in 1983. The Shoshone population was at 1768 m elevation and 38° 56' 22.43"N latitude, and was founded circa 1976 with an unknown number of individuals (Goodchild, unpublished). The Shoshone population is approximately 330 km north and 600 m higher in elevation than Lake Harriet, which contributes to, differences in air/water temperature and day lengths (Table 2.1). These differences in temperature may have important effects on the reproductive phenology and key life history characters.

2.3.2. Fish Collections

During each of three sample periods we collected up to 75 fish at each site in 2014 (mid-May, mid-June and early August). These dates are within the main reproductive season for poolfish in their native habitat (Deacon and Williams 2011). Baited Gee Minnow traps (1/4-inch mesh) were deployed and all adult captured fish were placed in approximately 8 l of water from which samples of up to 75 fish were haphazardly selected. Fish were euthanized with 500 mg/l MS-222 (Western Chemicals, Inc.), preserved in 10% formalin, and transported to the laboratory for processing.

2.3.3. Life History Measurements

Due to the limited number of males captured, the following life history descriptions focus only on females unless noted. The fish were dissected and all material from the alimentary tract was removed. Reproductive tissues and somatic tissues were then separated, and ovaries were teased apart and spread under a dissecting scope. Eggs were classified to developmental stage based on the oil droplet characteristics (Grier et al. 2009; Uribe 2012) and counted. Subsequently reproductive (*reproductive mass*) and somatic (gross *somatic mass*) tissues dried at 56° C for 48h and then separately weighed on a digital scale to the nearest 0.1 mg. Because of their small size, up to five mature eggs were weighed collectively and this mass was divided by the number of eggs weighed to characterize “typical” egg mass (Stockwell and Weeks 1999).

To extract fat, somatic tissues were placed in a 20 ml scintillation vial and soaked in anhydrous ether for 96h, which was drained and replaced every 24h (Reznick and Braun 1987). To facilitate drying and fat extraction of larger fish, we sectioned the carcasses between the caudal peduncle and vent and used two vials for fat extraction. Pilot studies determined fat loss reached an asymptote by 96h. Samples were subsequently oven-dried for 24h at 56 °C and re-weighed (*somatic net mass*). *Fat content* was computed as a percentage of mass allocated to fat ((gross mass)-(net mass)/(gross mass))*100. Likewise, *reproductive allocation* was computed as the percentage of total dry mass contained in gonads relative to the unextracted somatic mass (reproductive mass/ somatic gross mass)*100.

2.3.4. Statistical Analyses

ANOVAs and ANCOVAs (IBM SPSS Statistics, v. 22) were used to test for the direct and interactive effects of POPULATION (Lake Harriet vs. Shoshone) and COLLECTION DATE (May, June, and August) for each of the following life history traits: *average female size*, *fat content*, *reproductive allocation*, *number of mature eggs* and *typical egg size*. To account for the size of the fish, net somatic mass was used as a covariate for evaluating fat content. For the latter three reproductive traits, gross somatic mass was used as a covariate. If the covariate was not significant, it was removed from the final model. In cases where size did not overlap, we used percent to compare traits instead of the covariate. Population specific ANOVA and post hoc tests (LSD) were used to determine intra-population differences, and t-tests were used between populations within sampling periods. To meet assumptions of normality and homogeneity of variance, data were transformed by either $\log_{10}$ (weights and lengths) or the arcsine of the square root (percent). Experimental wise error rate was maintained at 0.05.

2.4. Results

2.4.1. Size

Poolfish sizes varied from a standard length of 17.07 to 79.17 mm, and we observed substantial sexual dimorphism in size, with females being considerably larger than males (t=19.02, p <0.001; Figure 2.2). Poolfish males varied in size from 17.07 to 43.99 mm, with a mean size of 34.01 ± 1.06 mm. By contrast, poolfish females varied in size from 30.10 to 79.17 mm with a mean size of 47.73 ± 0.96 mm. The sex ratio of sampled poolfish was female biased for all samples with the exception of Shoshone May (Table 2.2; Figure 2.2).

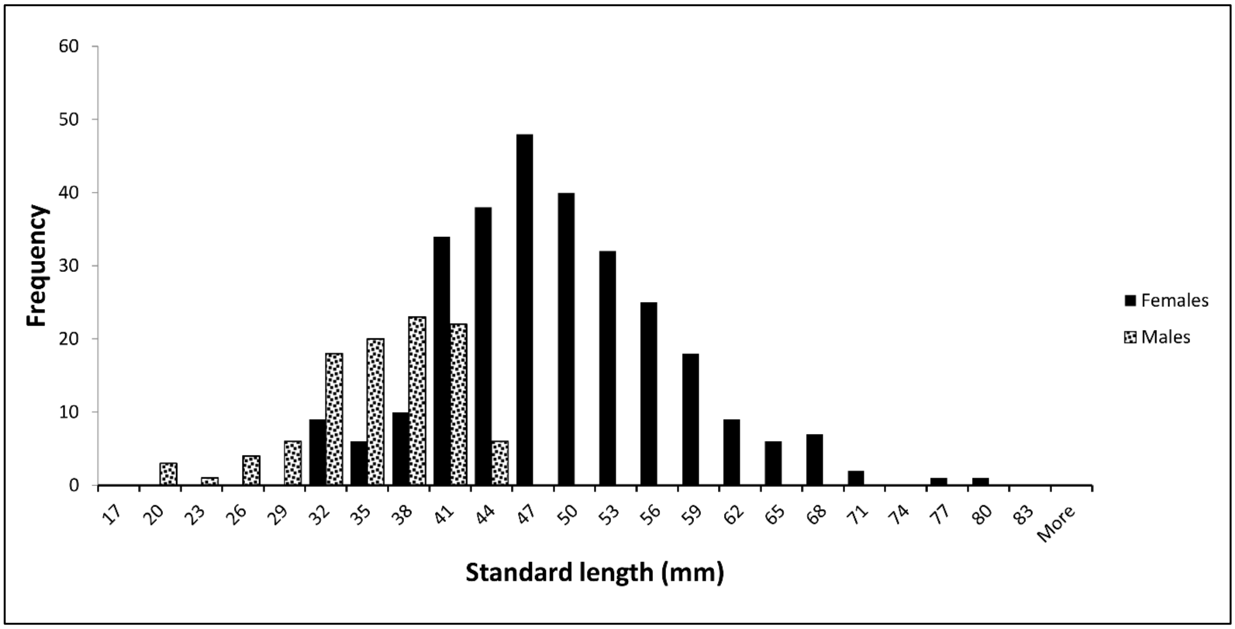

Figure 2.2: Length-Frequency distribution of males (stippled bars) and females (black bars)

Table 2.2: Numbers per gender of sampled Pahrump Poolfish.

Population	Date	Males	Females	Total	Percent Female
Shoshone	May	34	21	55	38.2
Shoshone	June	4	46	50	92.0
Shoshone	August	2	73	75	97.3
Lake Harriet	May	12	37	49	75.5
Lake Harriet	June	7	43	50	86.0
Lake Harriet	August	13	60	73	82.2

Size frequency histograms shows that Lake Harriet females were larger than Shoshone females for each sample period (Figure 2.3). The wide size distribution for Lake Harriet is suggestive of more cohorts than for Shoshone. Further, female standard length was significantly affected by POPULATION ($F_{1, 274} = 205.505$, $P < 0.001$) and by DATE ($F_{2, 274} = 6.008$, $P < 0.001$), but there was not a significant POPULATION X DATE affect ($F_{2, 274} = 1.056$, $P = 0.319$). Across all sample periods, Lake Harriet females were significantly longer (~20%) than Shoshone females, with no overlap of female lengths between sites (May [$t = 5.47$, $p < 0.001$], June [$t = 8.93$, $p < 0.001$], and August [$t = 11.12$, $p < 0.001$]; Figures 2.3 and 2.4). Two large individuals from Lake Harriet were deemed outliers and removed from further analysis.

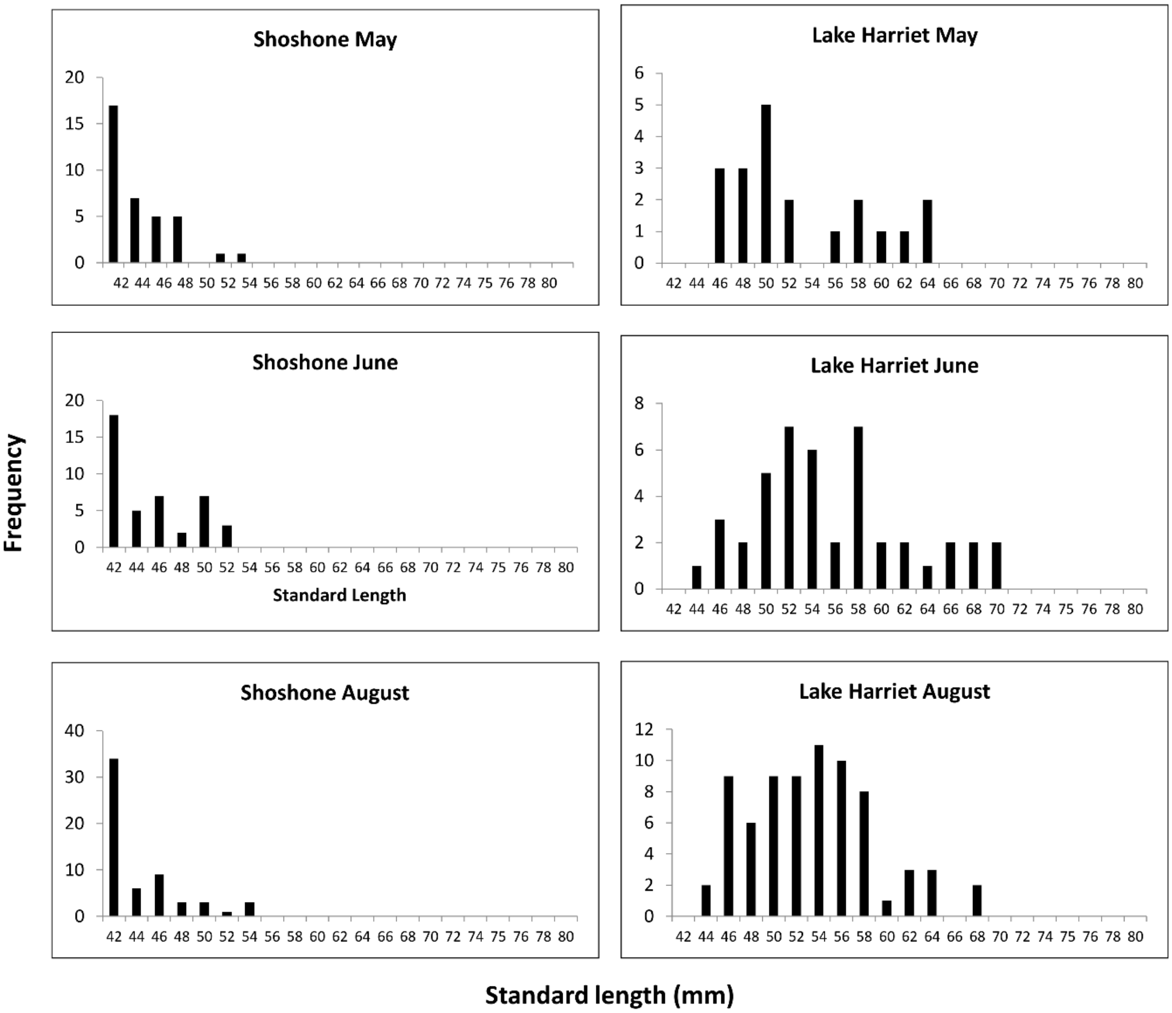

Figure 2.3: Length-Frequency distribution of Pahrump Poolfish females for temporal collections from Lake Harriet and Shoshone Pond, Nevada.

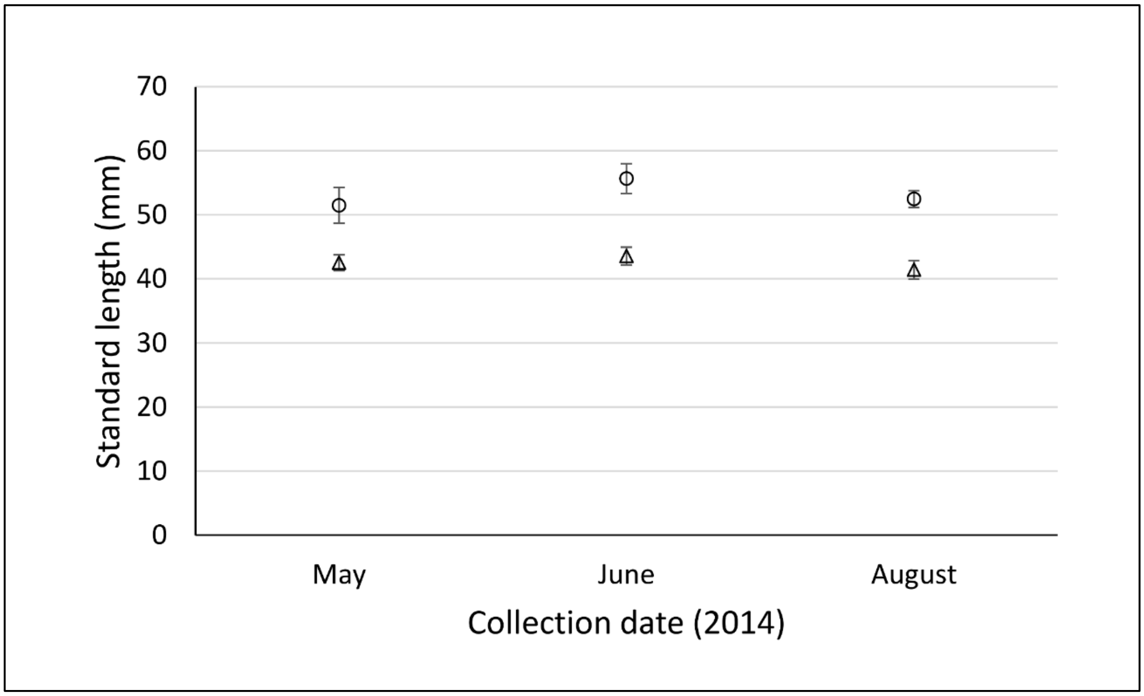

Figure 2.4: Average female standard length (Average ± SE) for each collection period for both Lake Harriet (o) and Shoshone (Δ).

Temporal variation in standard length was only observed among the Lake Harriet samples (F_2 =6.22, p = 0.016) which were significantly longer in June (55.68 ± 2.32 mm) compared to both May (51.50 ± 2.78 mm; p= 0.018) and August (52.46 ± 1.29 mm; p = 0.011), whereas the latter two samples did not differ (p = 0.557). For Shoshone, female standard length did not differ among the monthly samples (F_2 = 9.198, P = 0.094).

Female somatic mass (dry unextracted) was significantly affected by POPULATION ($F_{1, 274}$ = 328.74, P < 0.001), and DATE ($F_{2, 274}$ = 21.16, P < 0.001) but there was not a significant POPULATION X DATE interaction ($F_{2, 274}$ = 0.03, P = 0.287; Figure 2.5). Within each sample period, Lake Harriet females significantly larger than Shoshone females; May (t = 5.55, p < 0.001), June (t = 9.33, p < 0.001), and August (t = 12.00, p < 0.001; Figure 2.5).

For Lake Harriet, female mass did not significantly differ between June (1.02 ± 0.14 g) and August (0.89± 0.07 g; p = 0.062), but both were significantly larger than females collected in May (0.63 ± 0.10 g; p<0.001 and p = 0.006, respectively; Figure 2.5). For Shoshone, female mass did not significantly differ between June (0.39 ± 0.04 g) and August (0.41 +/- 0.04 g; p = 0.459), but both were significantly larger than females collected in May (0.29 ± 0.03 g; p = 0.001 and p < 0.001, respectively; Figure 2.5).

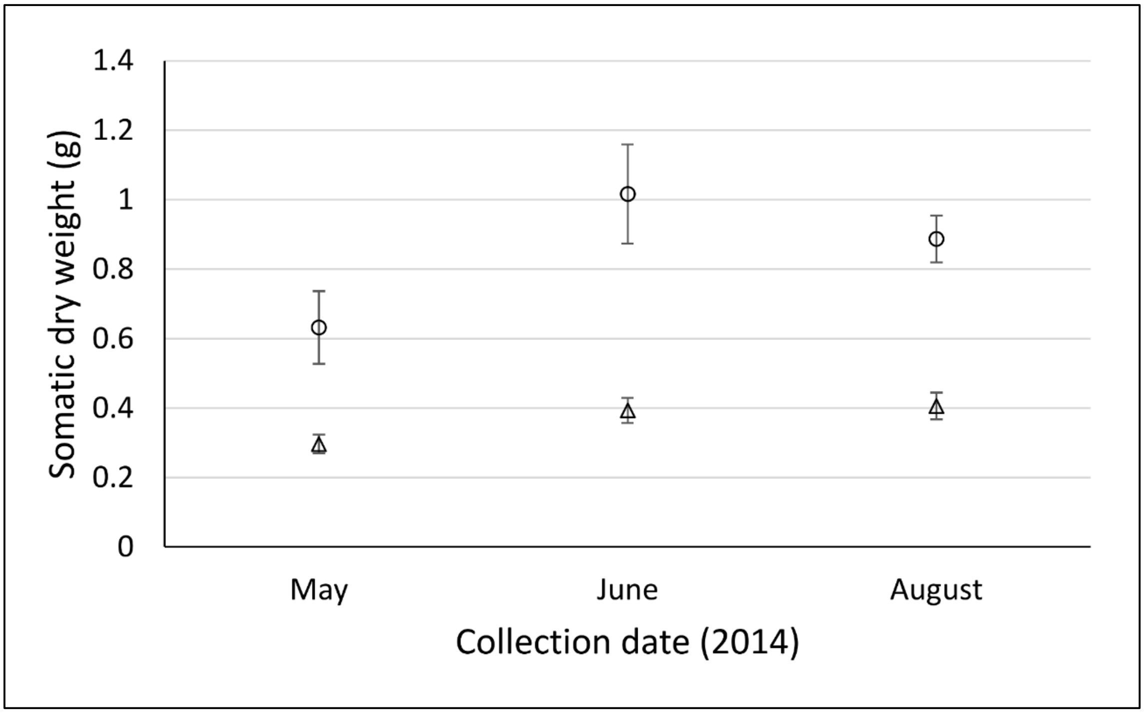

Figure 2.5: Female somatic mass (Average ± SE) is shown for each collection period for both Lake Harriet (o) and Shoshone (Δ).

2.4.2. Fat Content

Fat content was significantly affected by POPULATION ($F_{1, 274}$ =91.965, p < 0.001), DATE ($F_{2, 274}$ =52.796, p < 0.001), POPULATION X DATE ($F_{2, 273}$ =17.812, p < 0.001) and the covariate of extracted somatic mass ($F_{1, 273}$ =49.855, p < 0.001). Fat Content was significantly higher for Lake Harriet compared to Shoshone during May ($t = 3.71$, $p < 0.001$) and June ($t = 11.30$, $p < 0.001$), but not during August ($t = 1.43$, $p = 0.158$; Figure 2.6).

Fat content showed temporal variation within both populations. Within Lake Harriet, fat content varied across the breeding season ($F_2 = 15.907$, $p < 0.001$). Fat content did not differ between June (20.01 ± 1.06%) and August (21.50 ± 1.02%; $p = 0.176$), but both were significantly higher than May (15.85 ± 2.24%; $p < 0.001$ and $p < 0.001$, respectively; Figure 2.6). Within Shoshone, fat content also varied across the breeding season ($F_2 = 41.394$, $p < 0.001$), with significantly higher fat in August (19.63 ± 2.10%) compared to both the May (9.68 ± 1.99%; $p < 0.001$) and June (9.83 ± 1.39%; $p < 0.001$), while the May and June samples did not significantly differ ($p = 0.760$; Figure 2.6).

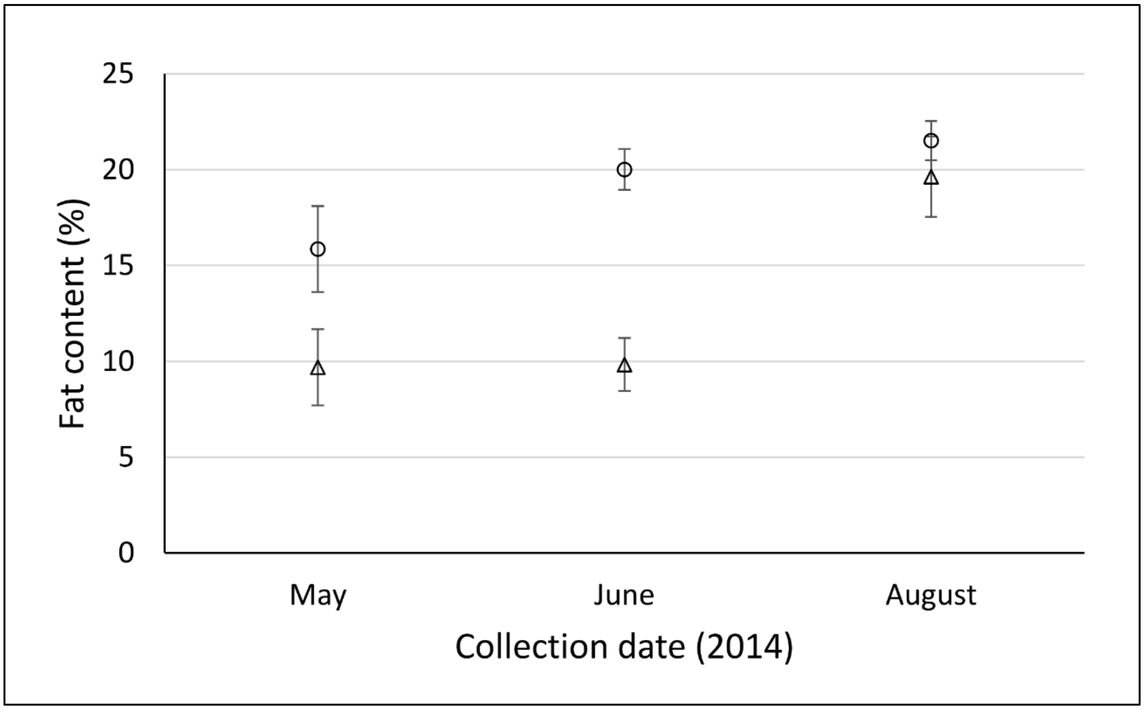

Figure 2.6: Average fat content (Average ± SE) is shown for each collection period for Lake Harriet (o) and Shoshone (Δ).

2.4.3. Reproductive Allocation

Reproductive allocation was significantly affected by POPULATION ($F_{1, 271}$ =28.420, $p < 0.001$), DATE ($F_{2, 271}$ =21.803, $p < 0.001$), and POPULATION X DATE ($F_{2, 271}$ =4.996, $p = 0.007$). Gross somatic mass was not a significant covariate and was not included in this final model. Reproductive allocation was significantly higher for Lake Harriet females compared to Shoshone females during June (12.27 ± 0.81 vs. 7.66 ± 0.85; t = 6.705, $p < 0.001$) and August (7.91.27 ± 0.52 vs. 6.36 ± 0.90; t = 2.458, $p = 0.016$), but not May (9.77 ± 1.29 vs. 8.08 ± 0.73; t = 1.171, $p = 0.251$; Figure 2.7a).

Reproductive allocation showed temporal variation within both populations. Within Lake Harriet, allocation significantly varied across all sampling periods (F_2 = 35.382, $p < 0.001$). Reproductive allocation was significantly higher during June (12.65 ± 0.83%) than either May (10.35 ± 1.37%; $p = 0.002$) or August (8.31 ± 0.53%; $p < 0.001$). May and August also significantly differed (p=0.004; Figure 2.7a). Within Shoshone, reproductive allocation also varied across the breeding season (F_2 = 5.517, $p = 0.005$), with significantly higher fat in May (9.18±0.75%) and June (8.33±0.88%; $p < 0.001$) than August (7.22±0.95%; $p = 0.002$ and 0.040 respectively), while the May and June samples did not significantly differ ($p = 0.251$; Figure 2.7a).

2.4.4. Egg Size and Production

The number of mature eggs per female was significantly affected by POPULATION ($F_{1, 260}$ =24.398, $p < 0.001$), DATE ($F_{2, 260}$ =52.796, $p < 0.001$), POPULATION X DATE ($F_{2, 260}$ =7.497, $p = 0.001$), and the covariate of gross somatic mass ($F_{1, 260}$ =51.445, $p < 0.001$). The number of mature eggs was significantly higher for Lake Harriet compared to Shoshone during

May ($t = 3.62$, $p < 0.001$), June ($t = 11.75$, $p < 0.001$), and August ($t = 10.37$, $p < 0.001$; Figure 2.7b).

Within both populations there was significant temporal variation in the number of eggs. Within Lake Harriet, the number of mature eggs per female varied across the breeding season ($F_2 =47.766$, $p < 0.001$). All dates were significantly different ($p < 0.001$), with approximately 30.9 ± 6.5 eggs in May, 87.9 ± 10.6 eggs in June, and 55.1 ± 4.4 eggs in August (Figure 2.7b). Within Shoshone, the number of mature eggs did not significantly differ between the May (17.1 ± 2.5), June (23.6 ± 3.0), and August samples (22.8 ± 4.2; $F_2 =3.001$, $p = 0.053$; Figure 2.7b).

Typical egg mass was not affected by POPULATION ($F_{1, 259} =0 .599$ $P =0.490$), DATE ($F_{2, 259} = 2.025$, $P = 0.134$), or POPULATION X DATE ($F_{2, 259} = 2.093$ $P = 0.125$). The mean typical egg size varied from 0.55 to 0.7 mg (Figure 2.7c). The covariate (gross somatic mass) was significant ($F_{1, 259} = 6.72$, $p = 0.010$). Egg size significantly differed between sites only in June ($t = 5.96$. $p < 0.001$; Figure 2.7c), with Lake Harriet eggs being heavier than Shoshone eggs (0.674 vs. 0.554 mg).

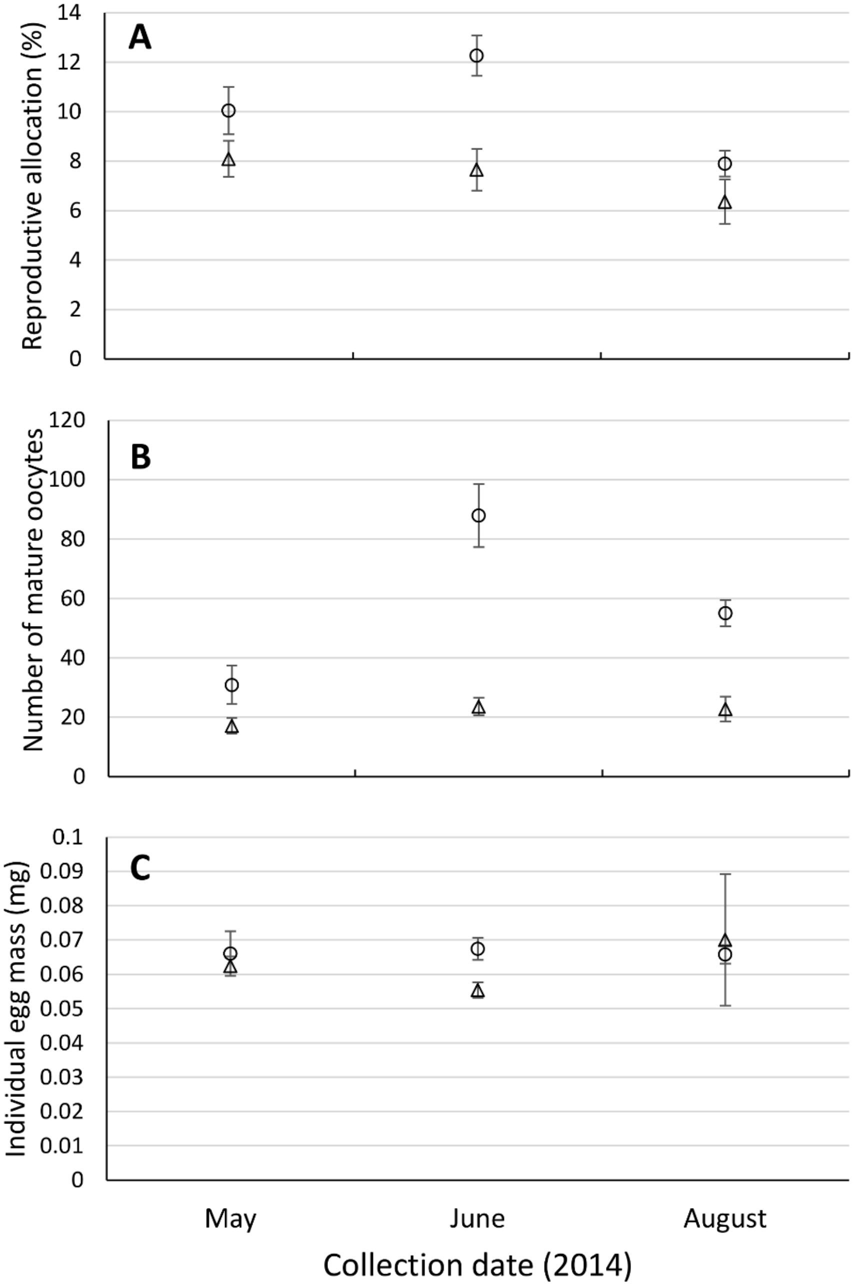

Figure 2.7: Reproductive traits (Average ± SE), including A) reproductive allocation, B) Number of mature eggs and C) typical egg size, for the three collections from Lake Harriet (o) and Shoshone (Δ).

2.5. Discussion

Pahrump Poolfish evolved in a small spring with limited environmental variation (La Rivers 1994; James Deacon, pers. comm.), but due to translocation this endangered species now occupies a diversity of habitats (Deacon and Williams 2011). Thus, conservation actions have provided a unique opportunity to evaluate phenotypic responses of this fish to these diverse habitats. We observed considerable phenotypic variation for Pahrump Poolfish life history characters between the two largest refuge populations. Body size divergence was particularly noteworthy with Lake Harriet females being about 2.3 times as large compared to Shoshone females. In general, the size distributions suggest more age cohorts, size limitation, and/or faster growth rates for Lake Harriet fish compared to Shoshone. Further, fat content, reproductive allocation and the number of mature eggs were all significantly higher for Lake Harriet females compared to Shoshone females. The typical size of eggs only differed in June with larger eggs for Lake Harriet females, thus, the increased number of eggs for Lake Harriet females did not reflect a life-history tradeoff of egg size versus egg number. Collectively, these findings suggest a correlation of the life history traits with body size. Such correlations are expected, since body size is generally positively correlated with reproductive characteristics in poikilotherms (Blueweiss et al. 1978).

Such phenotypic divergence may reflect evolutionary and/or plastic responses to different environmental conditions (i.e., Schluter 2001) such as genetic drift, differential predation pressure, temperature, and or fish density. It is unlikely that size selective predation of adults played a role as neither site had predatory fish, and piscivorous birds were rarely seen. By contrast, regardless of inflow temperature the pond water temperatures were considerably cooler at Shoshone compared to Lake Harriet due to the lower air temperatures resulting from the higher latitude and elevation of the Shoshone site (Table 2.1). For instance, the average monthly high temperature between May and August averages about 5 °C to 6 °C higher at Lake Harriet than at Shoshone Pond, and the minimum monthly temperature averages about 9 °C to 11 °C higher at Lake Harriet compared to Shoshone Pond. Consequently, water temperature loggers showed that Shoshone was consistently approximately 2 °C cooler than Lake Harriet throughout the year.

Differences in temperature patterns may also explain phenological differences for many life history traits (i.e., Wedekind and Küng 2010). Between May and June the Lake Harriet females showed dramatic increases in mass, reproductive allocation and the number of eggs while the Shoshone female exhibited modest changes during the same time period. Seasonal changes in Lake Harriet female fat content increased by June whereas Shoshone female fat content only increased in August. Collectively, these differences suggest that temperature may mediate growth patterns in Pahrump Poolfish, as has been reported for numerous other species (Vondracek et al. 1988; Jobling et al. 1995).

Density may also play a role in mediating poolfish life history traits. The approximate relative densities of poolfish (Kevin Guadalupe, pers. comm.) were about 26 times greater for Shoshone Stock pond (13 fish/m^3) compared to Lake Harriet (0.5 fish/m^3)(Table 2.1). Such effects may explain smaller sizes through self-thinning of the Shoshone females (Dunham and Vinyard 1997; White et al. 2007; Smith et al. 2013). It is also possible that unmeasured factors correlated with temperature and/or density may directly mediate poolfish life history traits, such as productivity. It is striking that the size variation between populations mimics the temporal variation in size at Manse Spring pre- and post-goldfish introduction at Manse Spring (Deacon and Williams 2011). Thus, life history responses may reflects responses to competition, intra-specific for our data, but inter-specific for the Manse Spring population.

The degree of divergence is especially striking given the history of these populations that included numerous bottlenecks (Figure 2.1). With these data, we are unable to distinguish whether the phenotypic responses reflect plasticity or an evolutionary response due to selection and/or genetic drift. However, even plastic responses of these populations have important management implications.

Our findings have relevance for selecting fish for establishing new populations. First, we observed pronounced sexual size dimorphism for poolfish from the refuge habitats. We also observed a general pattern female-skewed sex ratios, which is consistent with earlier work with both the Manse population (Deacon and Williams 2011) and the extant refuge populations (Heckmann 2009). Thus, to ensure that new populations are initiated with a suitable sex ratio, only young of year fish or a broad array of different sized fish should be used for stocking.

Population differences in life history traits may have important implications for restoration actions (e.g., Watters et al. 2003; Collyer et al. 2015). For instance, the phenotypes of the population would play an important role in future management of the species, primarily with the transfer of fish to other *ex situ* refuges for population augmentation or establishing new populations, as well as potential repatriation to their historic habitat. In fact, because they produce more eggs Lake Harriet genotypes/phenotypes are likely to have a numerical advantage over a similar number of Shoshone fish used in repatriation. In addition, such high variation in reproductive success would decrease the effective population size (Wright 1938; Nunney 1993).

Our work shows that refuge populations can rapidly diverge for important phenotypic traits, potentially presenting a phenotypic mismatch for reintroduction. Fish size is of particular importance due to the associated changes in other life history traits as well as the potential differences in top-down ecological impacts. Future work will be necessary to assess if the observed body size divergence reflects a plastic and/or an evolutionary response to divergent ecological conditions.

2.6. Acknowledgments

We thank M. Stockwell and H. Fairfield for assistance with field work; N. Westen for assistance with dissections; S. Parmenter (California Department of Fish and Game), J. Harter, L. Simons, C. Baldino and S. McKelvey (US Fish and Wildlife Service), J. Sjoberg, K. Guadalupe, and H. Korell (Nevada Department of Wildlife) and R. Keller (Nevada Division of State Parks), N. Beecher and J. Prieur (Southern Nevada Water Authority), N. Herms (Bureau of Land Management) and S. Henkanaththegedara, H. Hundt, J. Fisher, and K. McLean for logistical and field support. We also thank James Deacon and Harry J. Grier for constructive discussions regarding poolfish and S. Henkanaththegedara for revisions. This work has been conducted under California Scientific Collecting permit SC 9459, and USFWS permit TE126141-2, Nevada Scientific Collecting Permit S 34628 and NDSU IACUC #12027. This work was supported by a Nevada State Wildlife Grant administered by J. Sjoberg to C.A.S. and S.G., as well as stipend support for S.G. from the NDSU Environmental & Conservation Sciences Graduate Program.

2.7. References

Blueweiss, L., H. Fox, V. Kudzma, D. Nakashima, R. Peters, and S. Sams. 1978. Relationships between body size and some life history parameters. Oecologia 37: 257-272.

Collyer M. L., J. S. Heilveil, and C. A. Stockwell. 2011. Contemporary evolutionary divergence for a protected species following assisted colonization. PloS ONE 6: e22310.

Collyer, M. L., J. M. Novak, and C. A. Stockwell. 2005. Morphological divergence of native and recently established populations of White Sands Pupfish (*Cyprinodon tularosa*). Copeia 2005: 1-11.

Collyer, M. L., M. E. Hall, M. D. Smith, and C. W. Hoagstrom. 2015. Habitat-Morphotype Associations of Pecos Pupfish (*Cyprinodon pecosensis*) in Isolated Habitat Complexes. Copeia 2015: 181-199.

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.

Dunham, J. B., and W. L. Minckley. 1998. Allozymic variation in desert pupfish from natural and artificial habitats: genetic conservation in fluctuating populations. Biological Conservation 84: 7-15.

Dunham, J. B., and G. L. Vinyard. 1997. Relationships between body mass, population density, and the self-thinning rule in stream-living salmonids. Canadian Journal of Fisheries and Aquatic Sciences 54:1025-1030.

Grier, H. J., M. C. Uribe-Aranzábal, and R. Patiño. 2009. The ovary, folliculogenesis, and oogenesis in teleosts. Reproductive biology and phylogeny of fishes (agnathans and bony fishes) 8: 25-84.

Heckmann, R. A. 2009. The adaptive characteristics and parasitofauna of the three refugea populations of Pahrump poolfish, Empetrichthys latos latos (Miller), Nevada (USA). Proceedings of Parasitology 47: 1-32.

Hendrickson, D. A., and J. E. Brooks. 1991. Transplanting short-lived fishes in North American deserts: review, assessment, and recommendations. Pp 283-298. *In*: Battle Against Extinction. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

Hendry, A. P., and M. T. Kinnison. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. Evolution 1999: 1637-1653.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290(5491): 516-518.

Jobling, M, H. K. Johnsen, G. W. Pettersen, and R. J. Henderson. 1995. Effect of temperature on reproductive development in Arctic charr, Salvelinus alpinus (L.) Journal of Thermal Biology 20: 157–165.

Kinnison, M. T., and N. G. Hairston. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. Functional Ecology 21: 444-454.

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

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

Miller, R. R. 1969. Conservation of Fishes of the Death Valley System in California and Nevada. Transactions of the California-Nevada Section, The Wildlife Society: 107-122.

Minckley, W.L. 1995. Translocation as a Tool for Conserving Imperiled Fishes: Experiences in Western United States. Biological Conservation 72: 297-309.

Minckley, W. L., and J. E. Deacon. 1991. Western fishes and the real world: the enigma of "endangered species" revisited. Pp 405-413. *In*: Battle Against Extinction. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

Nunney, L. 1993. The influence of mating system and overlapping generations on effective population size. Evolution 47: 1329-1341.

Pister, E. P. 1993. Species in a bucket. Natural History 102: 14.

Reznick, D. N., and B. Braun. 1987. Fat cycling in the mosquitofish (*Gambusia affinis*): Fat storage as a reproductive adaptation. Oecologia 73: 401-413.

Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112: 183-198.

Reznick, D. N., L. Nunney, and H. Rodd. 2004. Empirical evidence for rapid evolution. Pp 101-118. *In* Evolutionary Conservation Biology. D. Couvet, U. Diekman, and R. Ferrier.(eds.). Cambridge University Press.

Roff, D. A. 2001. Life history evolution. Sunderland: Sinauer Associates. 527pp.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology and Evolution 16, 372-380.

Smith, J. A., L. J. Baumgartner, I. M. Suthers, D. S. Fielder, and M. D. Taylor. 2013. Density-dependent energy use contributes to the self-thinning relationship of cohorts. The American Naturalist 181: 331-343.

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

Stockwell, C. A., A. P. Hendry, and M.T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18: 94–101.

Uribe, M. C., H. J. Grier and L. R. Parenti. 2012. Ovarian structure and oogenesis of the oviparous goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (Teleostei, Cyprinodontiformes). Journal of Morphology 273:371–387.

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

Watters, J. V., S. C. Lema, and G. A. Nevitt. 2003. Phenotype Management: A New Approach to Habitat Restoration. Biological Conservation 112: 435-445.

Wedekind, C., and C. Küng. 2010. Shift of Spawning Season and Effects of Climate Warming on Developmental Stages of a Grayling (Salmonidae). Conservation Biology 24: 1418-1423.

White, E. P., S. K. M. Ernest, A. J. Kerkhoff, and B. J. Enquist. 2007. Relationships between body size and abundance in ecology. Trends in Ecology and Evolution 22:323–330.

Wilcox, J. L., and A. P. Martin. 2006. The devil's in the details: genetic and phenotypic divergence between artificial and native populations of the endangered pupfish (Cyprinodon diabolis). Animal Conservation 9: 316–321.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. Science 87:430-431.

CHAPTER 3. AN EXPERIMENTAL TEST OF NOVEL ECOLOGICAL COMMUNITIES OF IMPERILED AND INVASIVE SPECIES [1]

3.1. Abstract

Imperiled fish species are often managed by establishing refuge populations as a hedge against extinction, but suitable sites are often at a premium. Thus, managers may wish to consider novel management scenarios such as establishing multi-species refuges that may even already include undesirable species. To determine suitability of multi-species refuges, we established experimental communities that included allopatric and sympatric communities of three fish species: Endangered Pahrump Poolfish *Empetrichthys latos*, Amargosa Pupfish *Cyprinodon nevadensis*, and invasive Western Mosquitofish *Gambusia affinis*. Mosquitofish juvenile production was not significantly affected by the presence of other species (Mean ± SE: 50 ± 18 allopatric, 33 ± 6 with poolfish and 38 ± 7 with both poolfish and pupfish). Similarly, pupfish persisted in sympatry with both poolfish and mosquitofish, but pupfish had higher juvenile production when maintained in allopatry (557 ± 248) and in the presence of poolfish (425 ± 36) compared pupfish production in the presence of both poolfish and mosquitofish (242 ± 32). By contrast, poolfish juvenile production was high in allopatry (123 ± 17), but significantly reduced in the presence of pupfish (6.6 ± 1.2), in the presence of mosquitofish (1.0 ± 0.5), and in a community of all three species (0.5 ± 0.4). This suggests that translocated pupfish may coexist in refuges containing non-native mosquitofish, but the endangered poolfish

---

[1] The material in this chapter was co-authored by Shawn C. Goodchild and Craig A. Stockwell. Shawn C. Goodchild had the primary responsibility for collecting samples in the field and conducting the experiment. Shawn C. Goodchild was the primary developer of the conclusions described here within, drafted and revised all previous versions of this chapter. Craig A. Stockwell served as a proofreader and supplied constructive comments for an improved chapter. This chapter has been published in *Transactions of the American Fisheries Society* 145:264–268, 2016.

are not compatible with the other species and the current management of poolfish in single species refuges is appropriate. Consequently, our results indicate that multi-species refuges are suitable for some endangered species, which will give managers more latitude for the management of these imperiled species.

3.2. Introduction

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

In general, non-native species are detrimental to the persistence of imperiled desert fish (e.g. Meffe 1985; Marsh and Langhorst 1988). However, recent work has shown that impacts of invasive species may not be universally negative and degrees of compatibility may be condition specific, involving abiotic as well as biotic factors (Dunson and Travis 1991; Henkanaththegedara and Stockwell 2012, 2014). For instance, intraguild predation, the predation of potential competitors, may allow co-persistence among native and nonnative species alike (Lenon et al 2002; Henkanaththegedara and Stockwell 2012, 2014), thereby increasing management opportunities for protecting rare species. For instance, one intriguing option could be to establish multi-species refuges at sites that already harbor other native species or even non-native species (Mueller 2006). However, information will be required to evaluate the likelihood of co-persistence for the targeted species.

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

While appealing, multispecies refuges for desert fish have rarely been tested (though, see Robinson and Ward 2011). A first step to testing the multispecies concept is to understand species compatibility among key species. Here we examine compatibility based on species-specific juvenile production within experimental communities of Pahrump Poolfish, Amargosa Pupfish, and Western Mosquitofish raised in sympatry and allopatry. We performed this experiment in a semi-natural mesocosm using species and habitats that could be considered proxies for similar species and potential *ex situ* refuges, respectively. We hypothesize that poolfish or pupfish may coexist in multi-species communities.

3.3. Materials and Methods

Fish were wild caught from Spring Mountain Ranch State Park, Clark County, Nevada (poolfish), Crystal Spring, Nye County, Nevada and Little Alkali Spring, Mono County, California (mosquitofish), and River Springs, Mono County, California (pupfish *C. n. amargosae*). Allopatric and sympatric communities of poolfish, mosquitofish and pupfish were maintained using mesocosms at an outdoor field site on the North Dakota State University campus, Cass County, North Dakota, USA.

Experimental fish communities were assigned to circular 1211-liter rigid plastic tubs. Gravel substrate and artificial cover material (five 0.5m-long clumps of plastic mesh weighted to simulate rooted aquatic plants) were added in all tubs to create structure. These mesocosms were inoculated with a mixture of plankton from a local semi-permanent wetland, covered with wire mesh, aerated, and maintained at approximately 700 liters. The treatments included experimental fish communities that consisted of one, two, or all three species.

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

Water conditions and quality were monitored. The water temperatures changed relative to environmental conditions in a diel rhythm and over the course of the experiment. The experiment was terminated at 71 days at which time all fish were removed from the tubs, and euthanized with 500 mg/l of MS-222 (Western Chemical, Inc.). Fish were then preserved in 10% formalin, identified, sexed and counted. We recorded number of surviving juveniles per species per treatment as a measure of productivity. We also recorded the number of juveniles per surviving adult female for poolfish and pupfish, but not for mosquitofish as the final number of adult mosquitofish females could include both parental females as well as first generation adult females.

Treatment comparisons were analyzed (SPSS, IBM Corp.) using Kruskal-Wallis and the experimental wise error rate was maintained at 0.05 using sequential Bonferroni (Rice 1989).

3.4. Results

The mesocosms appeared to provide adequate environmental conditions for survival and reproduction. In many cases the number of adult mosquitofish adults exceeded the number of founders due to recruitment of first generation offspring. Thus, estimating mosquitofish adult survival was not possible. Average adult survival across the allopatric (single species) and sympatric (multiple species) treatments varied from 85% to 89% for poolfish and from 76% to 100% for pupfish (Table 3.1).

Table 3.1: Average survival of adult pupfish and adult poolfish per treatment.

Treatment	Poolfish Survival	Pupfish Survival
Allopatric Poolfish	89%	n/a
Allopatric Pupfish	n/a	100%
Poolfish & Pupfish	88%	94%
Poolfish & Mosquitofish*	86%	n/a
Poolfish & Pupfish & Mosquitofish*	85%	76%**

* Mosquitofish adult survival was not estimated because the final number of adult mosquitofish. Included both founding and first generation adults.
** If tank with only one pupfish survivor is excluded, average pupfish survival is 83.

The lower survival for pupfish was associated with one mesocosm with all three species that had only one surviving adult pupfish. We removed this tank for additional analyses of juvenile production of all three species. The final sex ratio for poolfish varied from 11% to 75%

female, but number of surviving poolfish females did not significantly differ among treatments (Kruskal-Wallis, H = 1.825, d.f. = 3, P = 0.609).

All three species successfully reproduced when in allopatry. The number of mosquitofish juveniles per mesocosm did not significantly differ among treatments (Kruskal-Wallis, H =0.578, d.f. = 2, *P* = 0.749). There were 253 ± 95 (Mean ± SE) mosquitofish juveniles in allopatry, compared to 180 ± 31 mosquitofish juveniles when sympatric with only poolfish, and 187 ± 27 mosquitofish juveniles when sympatric with both poolfish and pupfish (Figure 3.1).

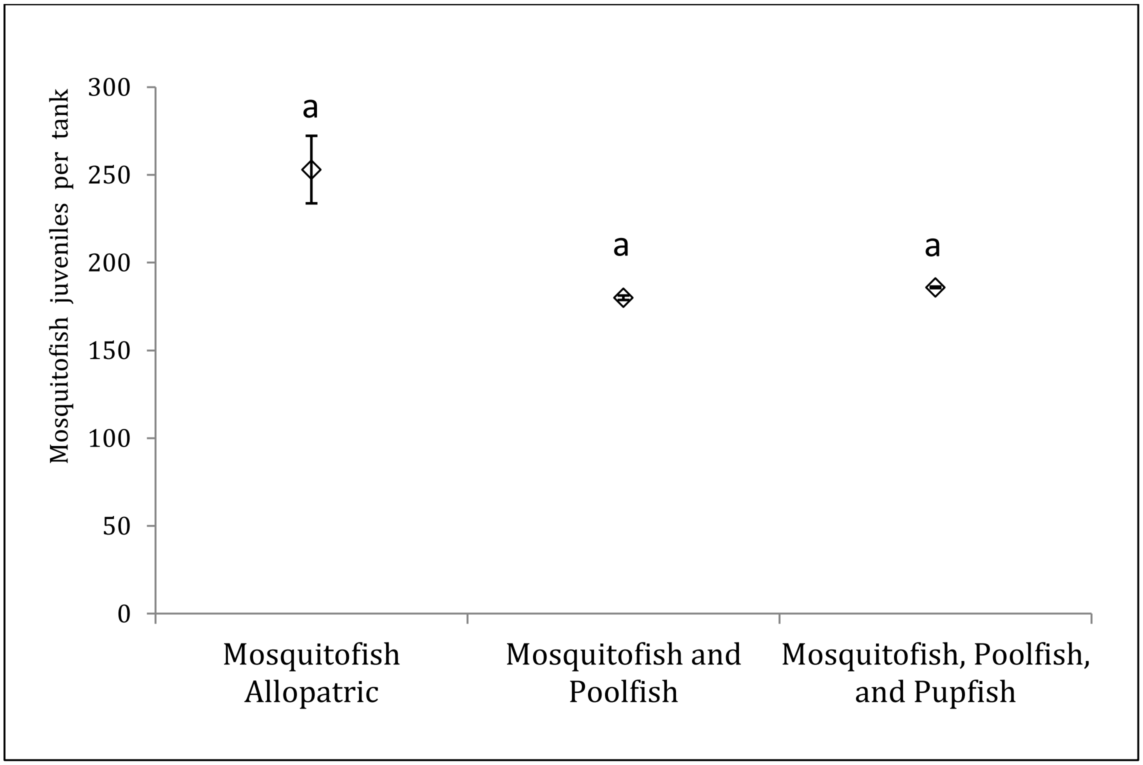

Figure 3.1: Number of Mosquitofish juveniles per tank by treatment.

The number of pupfish juveniles in allopatry (Mean ± SE: 557 ± 248) and when sympatric with poolfish (425 ± 36) did not differ, but both were significantly higher compared to pupfish in the presence of both poolfish and mosquitofish (242 ± 32) (Kruskal-Wallis, H =8.87, d.f. = 2, *P* = 0.012) (Figure 3.2a). However, pupfish juvenile production per female did not differ among the three treatments (Kruskal-Wallis, H =1.032, d.f. = 2, *P* = 0.597; Figure 3.2b)

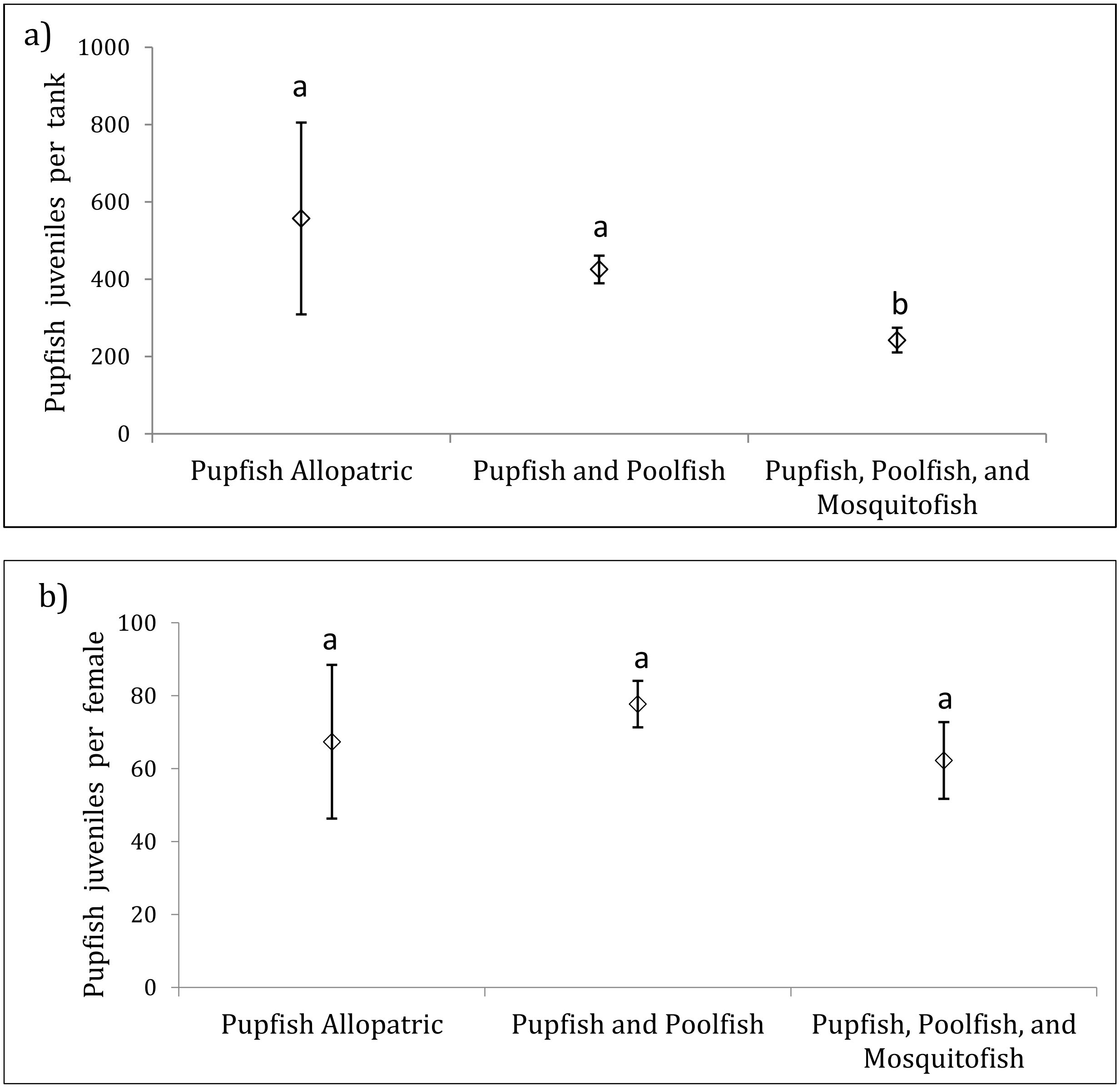

Figure 3.2: a) Number of Pupfish juveniles, and b) number of pupfish juveniles per female, per tank by treatment.

Poolfish sympatric with pupfish and/or mosquitofish were severely limited in recruitment. The number of poolfish juveniles was significantly higher in allopatry (Mean ± SE: 123 ± 17) than when sympatric with pupfish (7 ± 1), sympatric with mosquitofish (1 ± 0.5), and sympatric with both species (0.5 ± 0.4) (Kruskal-Wallis, H = 26.591, d.f. = 3, *P* = 0.000), but there were not any significant differences in poolfish juvenile production among the three sympatric communities (Figure 3.3).

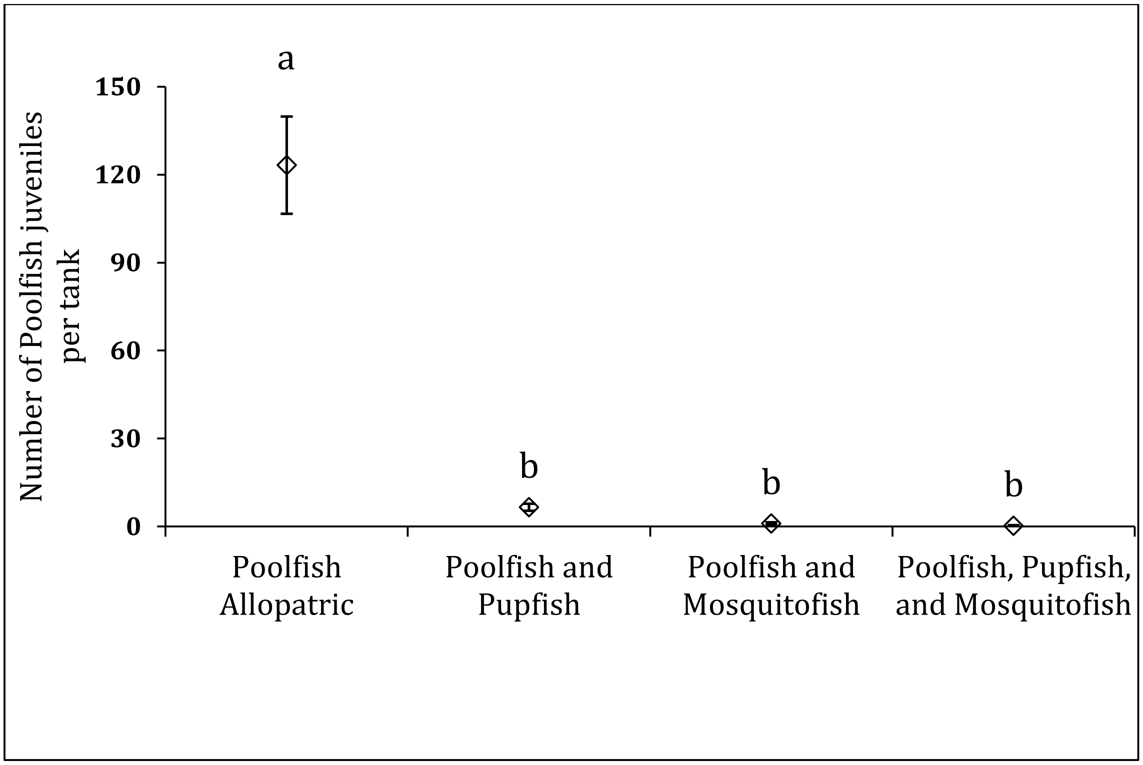

Figure 3.3: Number of Poolfish Juveniles per tub by treatment.

The number of poolfish juveniles per female was significantly higher in allopatry (55 ± 16) than when sympatric with pupfish (3 ± 0.7), sympatric with mosquitofish (0.6 ± 0.3), and sympatric with both pupfish and mosquitofish (0.25 ± 0.2) (Kruskal-Wallis, H = 25.104, d.f. = 3,

$P < 0.001$) (Figure 3.4). There were not any differences in poolfish juveniles per female among the three sympatric communities.

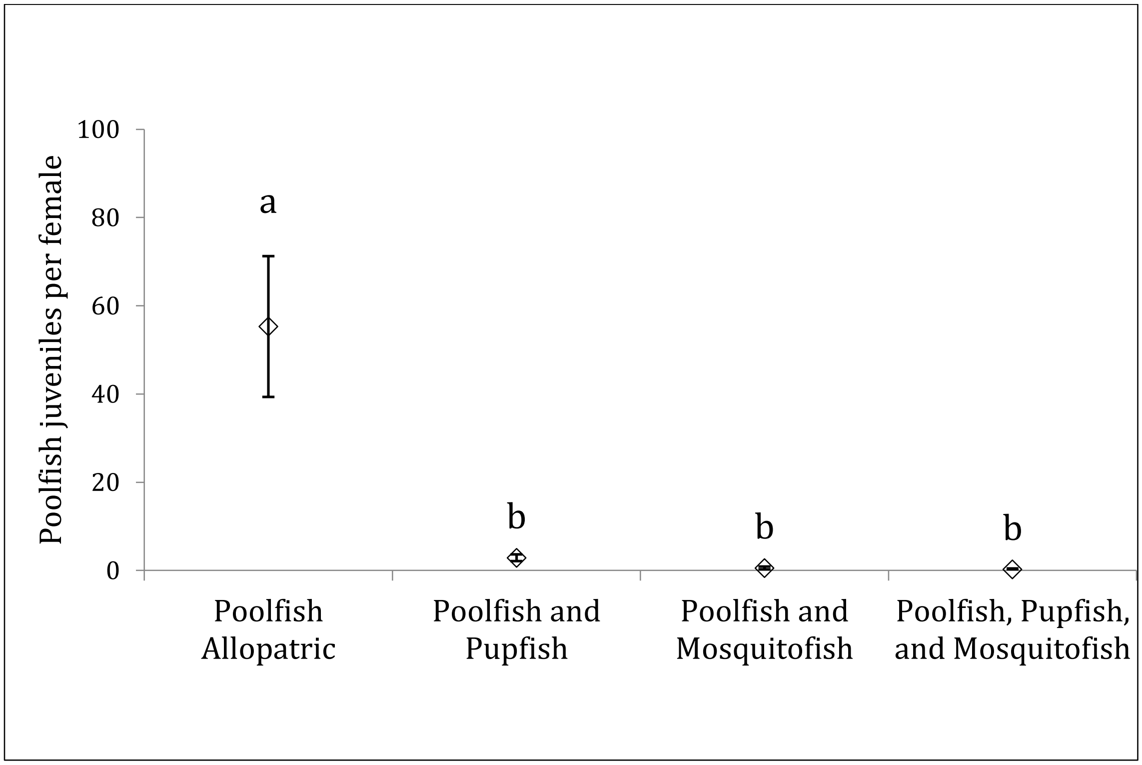

Figure 3.4: Number of Poolfish juveniles per female by treatment.

3.5. Discussion

Novel multi-species refuges are an appealing solution to the challenge of protecting species when refuge habitats are at a premium. Such an approach will require that all species of concern can co-persist; however, the findings from this experiment suggest differences among species in their potential to co-persist with other fish species.

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

In contrast to pupfish, poolfish were not able to establish in the presence of pupfish and/or mosquitofish. The habitats were sufficient to support high poolfish production when allopatric; however, poolfish juvenile survival was virtually zero when in the presence of pupfish and/or mosquitofish. Reduced poolfish juvenile survival was presumably due to predation on eggs and/or larvae, rather than competition, because food was provided.

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

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

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

Our findings, however, suggest that pupfish may be able to co-persist with mosquitofish. As such, labor-intensive removal of mosquitofish may not be necessary to establish and maintain pupfish refuge populations. Finally, our results suggest that novel multi-species refuges may expand conservation options and this concept deserves further exploration.

3.6. Acknowledgments

We thank S. Henkanaththegedara, S. Skinner, H. Hundt, J. Fisher, K. McLean, M. Stockwell and H. Fairfield for assistance with field work; S. Parmenter (California Department of Fish and Game), J. Harter, L. Simons, C. Baldino and S. McKelvey (US Fish and Wildlife

Service), J Sjoberg and K. Guadalupe, (Nevada Department of Wildlife), and R. Keller (Nevada Division of State Parks) for logistical and field support; and S. Henkanaththegedara for reviewing an earlier version of this manuscript. This work has been conducted under California Scientific Collecting permit SC 9459, USFWS permit TE126141-3, Nevada Scientific Collecting Permit S-34628 and NDSU IACUC #A12027. This work was supported by a Nevada State Wildlife Grant administered by J. Sjoberg to C.A.S. and S.G., as well as stipend support for S.G. from the NDSU Environmental and Conservation Sciences Graduate Program. We dedicate this paper to the late Dr. Jim Deacon, a pioneer in the study and conservation of desert fishes.

3.7. References

Baugh, T. M. and J. E. Deacon. 1988. Evaluation of the role of refugia in conservation efforts for the Devils Hole pupfish, *Cyprinodon diabolis* Wales. Zoo Biology 7: 351-358.

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. 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.

Dunham, J. B., and W. L. Minckley. 1998. Allozymic variation in desert pupfish from natural and artificial habitats: genetic conservation in fluctuating populations. Biological Conservation 84: 7-15.

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 (Suppl. 1): 218–231.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245: 477-480.

Hendrickson D. A. and A. V. Romero. 1989. Conservation status of desert pupfish, *Cyprinodon macularius*, in México and Arizona. Copeia: 478-483.

Henkanaththegedara, S. M. and C. A. Stockwell. 2012. 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.

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.

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

Lenon, N., K. Stave, T. Burke, and J. E. Deacon. 2002. Bonytail (*Gila elegans*) may enhance survival of razorback sucker (*Xyrauchen texanus*) in rearing ponds by preying on exotic crayfish. Journal of the Arizona-Nevada Academy of Science 34: 46-52.

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

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 Publication of the Museum of Zoology at the University of Michigan 68: 1-155.

Miller, R. R., and E. P. Pister. 1971. Management of the Owens pupfish, *Cyprinodon radiosus*, in Mono County, California. Transactions of the American Fisheries Society 100: 502-509.

Minckley, W. L. 1995. Translocation as a tool for conserving imperiled fishes: Experiences in western United States. Biological Conservation 72: 297-309.

Moyle P. B. and G. M. Sato. 1991. On the design of preserves to protect native fishes. Pp 155-169. *In*: Battle Against Extinction. W. L. Minckley and J. E. Deacon (eds.). University of Arizona Press, Tucson.

Mueller, G. A. 2006. Ecology of bonytail and razorback sucker and the role of off-channel habitats in their recovery. U. S. Geological Survey Scientific Investigation Report. 2006-5065.

Olden, J. D., M. J. Kennard, J. J. Lawler, and N. L. Poff. 2011. Challenges and opportunities in the implementing Managed Relocation for Conservation of Freshwater Species. Conservation Biology 25: 40-47.

Ostermann, S. D., J. R. Deforge, and W. D. Edge. 2001. Captive breeding and reintroduction evaluation criteria: a case study of peninsular bighorn sheep. Conservation Biology 15: 749–760.

Pister, E. P. 1993. Species in a bucket. Natural History 102: 14.

Pyke, G. H. 2005. A Review of the Biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries 15: 339-365.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Robinson, A. T., and D. L. Ward. 2011. Interactions between desert pupfish and gila topminnow can affect reintroduction success. North American Journal of Fisheries Management 31: 1093-1099.

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.

Soltz, D. L., and R. J. Naiman. 1978. The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, Science Series 30. 76pp.

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

USFWS. 1990. Recovery plan for the endangered and threatened species of Ash Meadows, Nevada. U.S. Fish and Wildlife Service, Portland, Oregon. 123 pp.

Wolf, C. M., B. Griffith, C. Reed, and S. A Temple. 1996. Avian and mammalian translocations: Update and reanalysis of 1987 survey data. Conservation Biology 10: 1142–1154.

CHAPTER 4. EFFECTS OF TWO SOURCE POPULATIONS OF MOSQUITOFISH ON PAHRUMP POOLFISH (*EMPETRICHTHYS LATOS*) AND AMARGOSA PUPFISH (*CYPRINODON NEVADENSIS*)

4.1. Abstract

Translocation is frequently used for the preservation of imperiled aquatic species; however, alien invasive species such as the Western mosquitofish (*Gambusia affinis*) often hinder management. It is unclear if these mosquitofish are generally detrimental or if there is population-specific variability in their potential to harm sensitive species. To detect potential differences, we established experimental communities that included allopatric and sympatric communities of the endangered Pahrump poolfish (*Empetrichthys latos*) or Amargosa pupfish (*Cyprinodon nevadensis*) with Western mosquitofish from two different source populations (Garrett and Wabuska Springs) that differed phenotypically in female body size.

Poolfish juveniles had higher survival in allopatry (36 ± 7.6) than in the presence of Garrett (2 ± 1.3) or Wabuska Gambusia (1 ± 0.7), but the latter two treatments did not differ. Pupfish juvenile survival was different among all three treatments, with higher survival in allopatry (503 ± 140) than in the presence of Garrett (185 ± 30), which in turn was higher than when in the presence of Wabuska Gambusia (119 ± 27). Pupfish juvenile production did not significantly differ between the two sympatric treatments ($U = 2.111$, $P = 0.555$). These results suggest that both poolfish and pupfish were universally repressed by Mosquitofish; although, poolfish were functionally extirpated, pupfish juveniles still maintained substantial survival. Further, there was a trend for pupfish to produce more juveniles when sympatric with a mosquitofish population comprised of larger fish than when sympatric with a population

comprised of smaller body fish. Thus, it appears that source population may be important in mediating impacts of mosquitofish on pupfish.

4.2. Introduction

Negative effects of invasive species are expected to be greater on native populations that evolved in simple communities than in more complex systems (Miller 1961; Courtenay and Deacon 1983; Cox and Lima 2006). In fact, invasive species have consistently had impacts on native fishes that evolved in simple communities (e.g. Meffe 1985; Marsh and Langhorst 1988; Lowe 2000). However, recent work has shown cases where native species co-persisted with non-natives for decades suggesting that the effects of alien species may be context-specific (Henkanaththegedara and Stockwell 2012; 2014), and vary due to abiotic as well as biotic factors (Dunson and Travis 1991).

Interactions between native and non-native species may be mediated by phenotypic variation within and among the interacting species. For instance, native Mohave tui chub adults are sufficiently large to prey on the adults of non-native mosquitofish and such predation appears to dampen reciprocal predation of mosquitofish on tui chub larvae (Henkanaththegedara and Stockwell 2013, 2014).

Phenotypic differences in body size also occur among populations of invasive species, which may mediate impacts of non-native species on native species. In fact, body size can differ by two-fold among non-native populations of poeciliid fishes (Stearns 1983; Reznick et al 1990; Stockwell & Vinyard 2000). Such phenotypic variation may mediate the impacts of invasive species on native species as well as the invaded ecosystems (Palkovacs et al. 2009). For example, vulnerability of native species to invasive predators may be mediated by gape size limitation as well as behaviors of the prey and predators (e.g. Nilsson and Bronmark 2000; Henkanaththegedara and Stockwell 2012; Goodchild and Stockwell in press).

Here, we examine if the impacts of mosquitofish populations on native species is context specific. We utilized two different stocks of mosquitofish that evolved differences in body size (Stockwell and Weeks 1999; Stockwell and Vinyard 2000) to examine impacts on two imperiled species. Previous work showed contemporary evolutionary body size divergence among four non-native mosquitofish populations introduced to Nevada in the 1940's (Stockwell and Weeks 1998; Stockwell and Vinyard 2000). We re-examined two of these mosquitofish populations in 2013 and found that females from the Garrett population were about two times the mass of females from the Wabuska population.

We experimentally examine the impacts of these two mosquitofish populations on two western fishes of conservation concern: The Amargosa pupfish (*Cyprinodon nevadensis amargosae*) and the Pahrump poolfish (*Empetrichthys latos*). An earlier experiment showed that mosquitofish had very strong impacts on experimental populations of Pahrump poolfish, but more modest impacts on experimental populations of Amargosa pupfish (Goodchild and Stockwell in press; Chapter 3). It is unclear if *Gambusia affinis* are generally noxious to these fish, or if effects may differ depending on the phenotypes of the mosquitofish. To test this we established two complimentary mesocosm experiments. In the first experiment, we compared the impacts of Garrett mosquitofish to the impacts of Wabuska mosquitofish on Pahrump poolfish. In the second experiment, we compared the impacts of the two mosquitofish populations on Amargosa pupfish. We hypothesize that mosquitofish of differing size phenotypes would differ in their effects on poolfish or pupfish in sympatry.

4.3. Material and Methods

Poolfish were wild caught from Spring Mountain Ranch State Park, Clark County, Nevada and Pupfish from River Springs, Inyo County, California; whereas, Western mosquitofish (MF) were captured from Garrett Spring (Garrett), Pershing Co. and Wabuska Spring (Wabuska), Lyon Co., Nevada. Allopatric and sympatric communities were established in two complementary experiments conducted using mesocosms at a field site on the North Dakota State University campus, Cass County, North Dakota, USA.

Experimental fish communities were established in circular 1211-liter rigid plastic tubs. Gravel substrate and artificial cover material (Five 0.5m-long clumps of plastic mesh weighted to simulate aquatic plants) were added uniformly in all tubs to create breeding substrate and habitat structure. Tubs were outdoors and subjected to ambient weather from May 12, 2014. The mesocosms were inoculated with a mixture of plankton from a local semi-permanent wetland, covered with wire mesh, aerated, and maintained at approximately 700 liters. Water conditions and quality were monitored. The water temperatures changed relative to environmental conditions in a diel rhythm and over the course of the experiment. To limit competition and comply with IACUC requirements, fish were fed daily of a mixture of aquarium flake and crushed koi pellets in a quantity equivalent to 5% of their stocked mass.

For the poolfish experiment, we established nine replicates of the following experimental communities: *i) allopatric poolfish*, *ii*) *poolfish and Garrett mosquitofish*, *iii*) *poolfish and Wabuska mosquitofish.* To obtain additional insights on responses of mosquitofish, we established three replicates of allopatric communities: *iv*) allopatric Garrett mosquitofish and *v*) allopatric Wabuska mosquitofish. Tanks were established with nine adult poolfish and/or nine gravid female mosquitofish. The initial sex ratio was unknown for poolfish, because it is difficult to determine the sex in this species. Because mosquitofish males are exceptionally small and have low survival, we used only gravid females.

For the pupfish experiment, we established nine replicates of the following experimental communities: *i) allopatric pupfish, ii) pupfish and Garrett mosquitofish, and iii) pupfish and Wabuska mosquitofish.* To obtain additional insights on responses of mosquitofish, we established three replicates of allopatric communities: *iv*) allopatric Garrett mosquitofish and *v*) allopatric Wabuska mosquitofish. Tanks were established seven adult pupfish (2 males and 5 females) and/or seven gravid female mosquitofish. The smaller starting population numbers for this experiment were necessary due to a limited number of available pupfish.

Poolfish and pupfish were stocked on June 3 to allow eggs to be laid and incubated, and mosquitofish were stocked on June 14, 2014. The experiment was terminated on July 19 (46 days for poolfish/pupfish and 35 days for mosquitofish) at which time all fish were removed from the tubs, euthanized with 500 mg/l of MS-222 (Western Chemical, Inc.) (NDSU IACUC # A12029), preserved in 10% formalin, and then counted.

Treatment comparisons of the number of juveniles produced per species per tank were analyzed using Kruskal-Wallis with post-hoc pairwise Mann-Whitney U tests and the experimental wise error rate was maintained at 0.1 (SPSS, IBM Corp.). All statistical analyses are conducted within each experiment, but we also compare and contrast the outcomes for the two experiments.

4.4. Results

The mesocosms appeared to provide adequate environmental conditions for survival and reproduction of all groups, as adult survival was universally high and all groups successfully produced juveniles when in allopatry.

4.4.1. Poolfish Experiment

The number of poolfish juveniles per population differed significantly among treatments ($H_2 = 18.002$, $P < 0.001$). There was significantly greater poolfish juvenile production when in allopatry (34 ± 8; mean number of juveniles/tank ± standard error) than when sympatric with Garrett mosquitofish (2 ± 1; $U = 11.944$, $P < 0.001$) or when sympatric with Wabuska mosquitofish (1 ± 1; juvenile/tank; $U = 14.056$, $P < 0.001$; Figure 1a). Poolfish juvenile production did not significantly differ between the two sympatric treatments ($U = 2.111$, $P = 0.555$) (Figure 4.1).

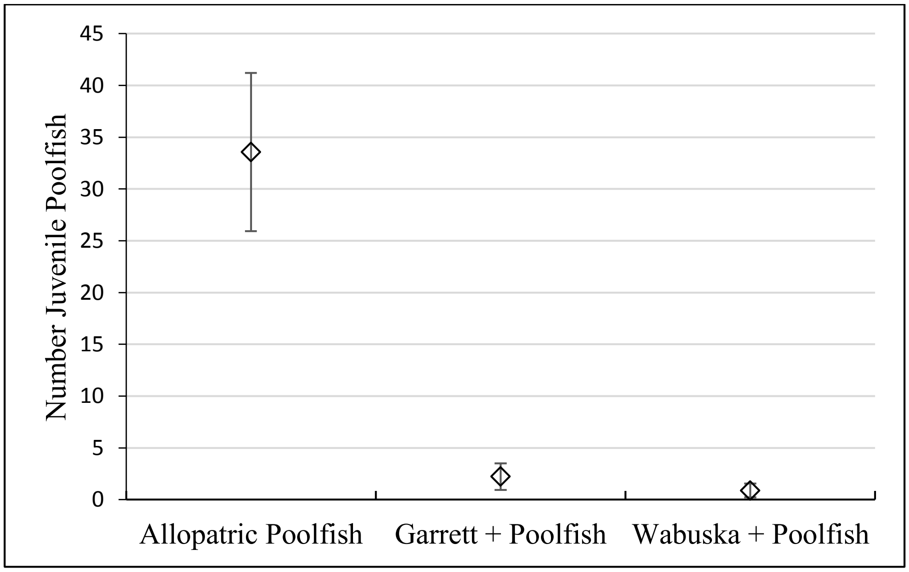

Figure 4.1: Average number of juvenile poolfish per population for each of the treatments.

Within the poolfish experiment, there were significantly more juvenile Garrett mosquitofish produced when in allopatry (160.7 ± 33.0; mean number of juveniles/tank ± standard error) than when Garrett mosquitofish were sympatric with poolfish (108 ± 13.8; U = 12.444, P = 0.008), 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) (U=-7.111, P = 0.131; Figure 4.2).

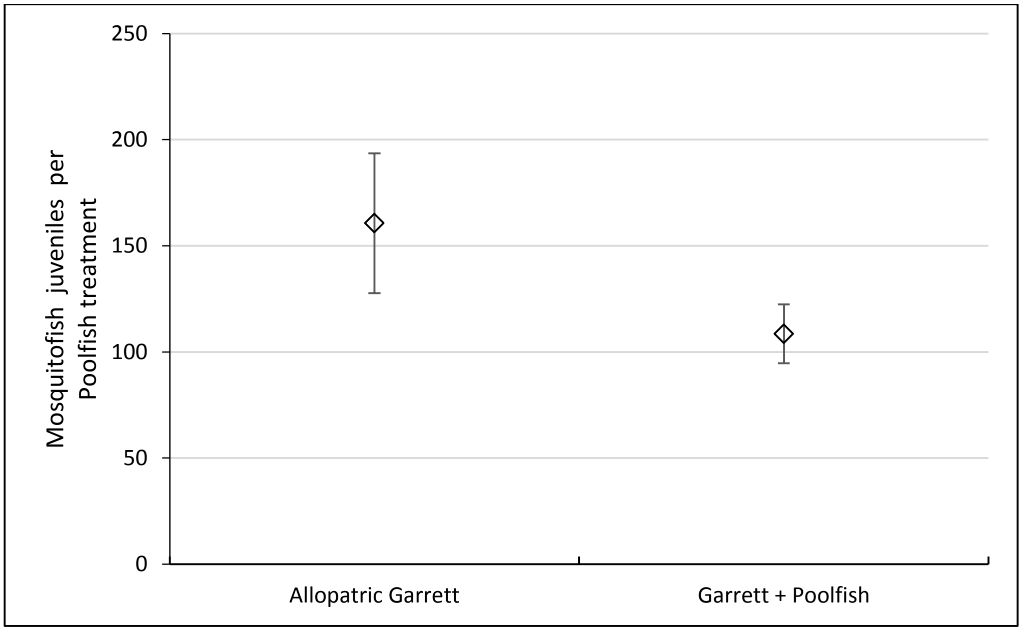

Figure 4.2: Average number of Garrett juvenile mosquitofish per population in allopatry and when sympatric with poolfish. Garrett was significantly greater in allopatry (U = 12.444, P = 0.008).

4.4.2. Pupfish Experiment

Numbers of Juvenile pupfish significantly varied among treatments ($H_2 = 7.858$, p = 0.020) (Figure 3.4). The number of juvenile pupfish produced was significantly higher for pupfish in allopatry (503 ±140; mean number of juveniles/tank ± standard error) than the sympatric communities with either Garrett mosquitofish (185 ± 30) (U = 5.333, p = 0.092) or

Wabuska mosquitofish (119 ± 27) (U = 8.833, p = 0.005), but the two sympatric treatments did not differ (U = 3.5, p = 0.175) (Figure 4.3).

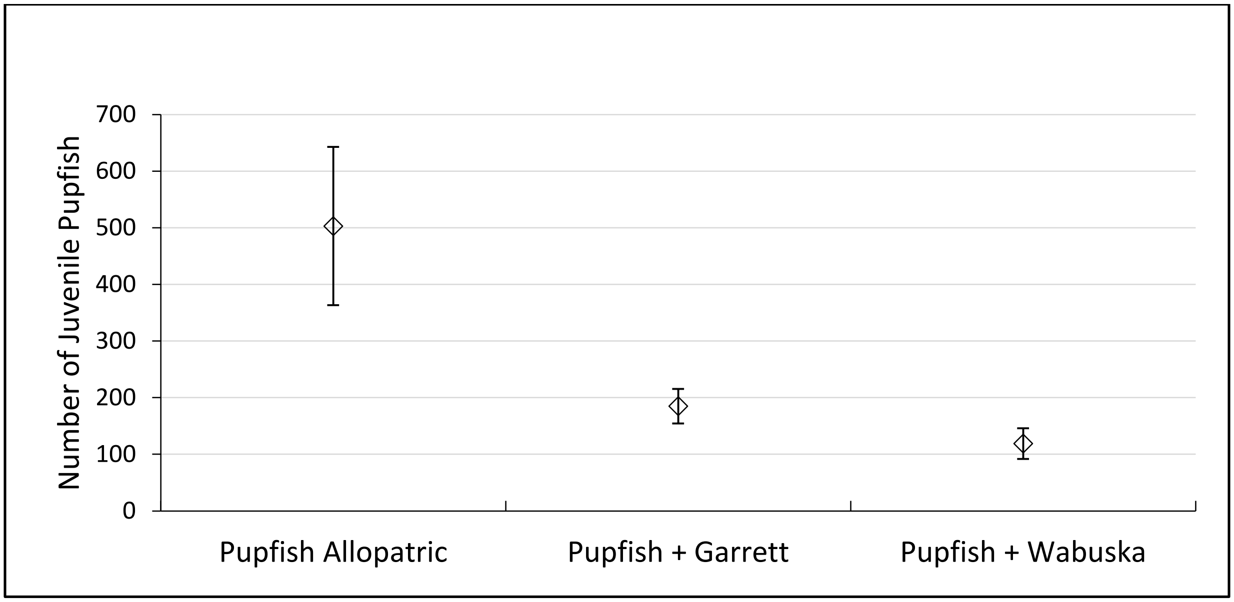

Figure 4.3: Average number of juvenile pupfish per treatment.

The number of juvenile Garrett mosquitofish produced in allopatry was not significantly different from juvenile production when sympatric with pupfish (U = 2.402, p = 0.121; Figure 4.4). Similarly, the number of juvenile Wabuska mosquitofish when in allopatry was not significantly different from juvenile production when sympatric with pupfish (U = 2.400, p = 0.121; Figure 4.5). Because brood size co-varies with mosquitofish female body size (Stockwell and Weeks 1999), we did not compare juvenile productivity between the two mosquitofish populations.

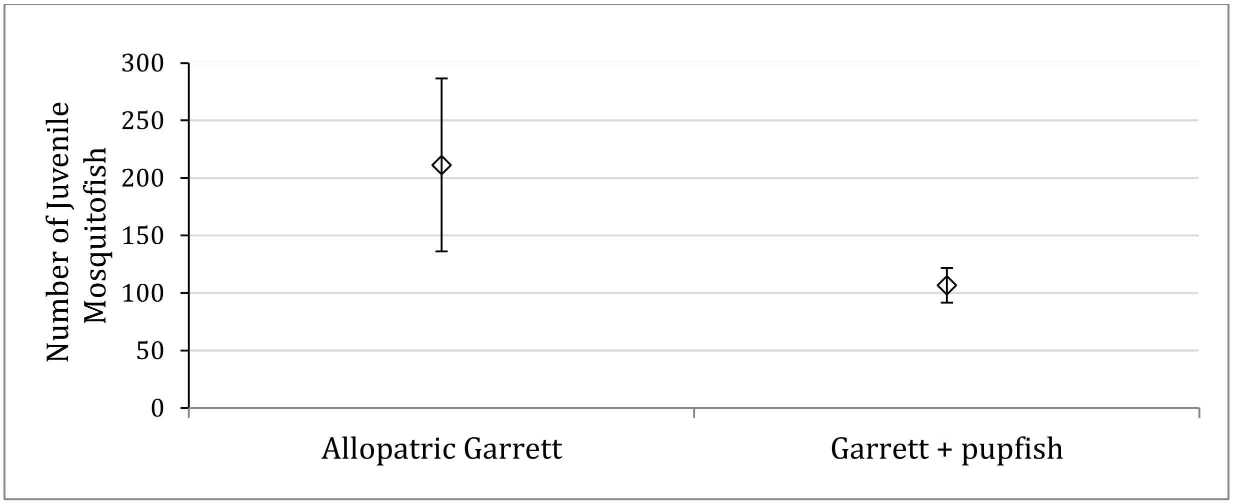

Figure 4.4: Number of juvenile mosquitofish per treatment within the Pupfish experiment.

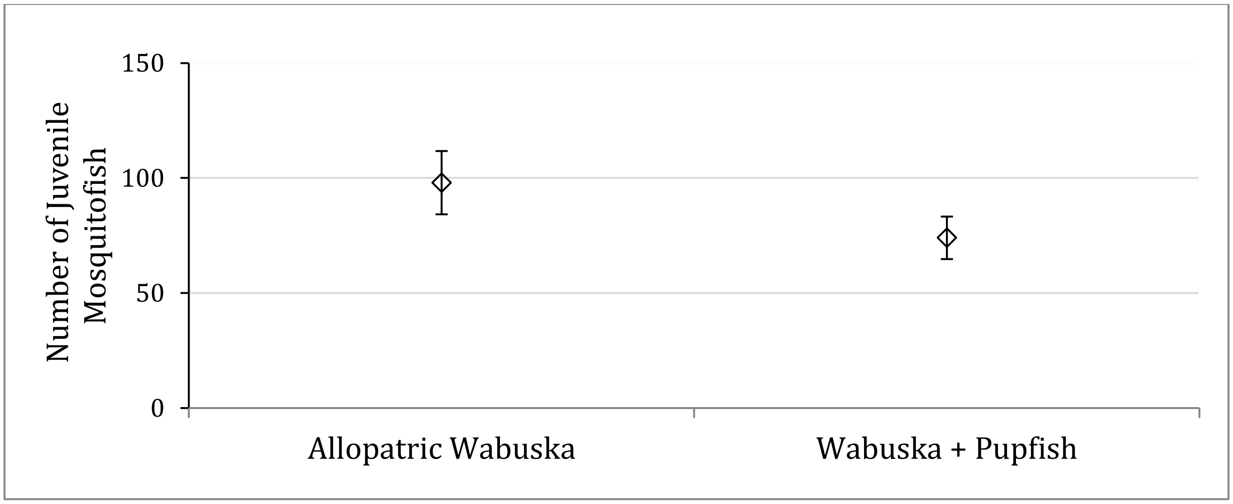

Figure 4.5: Number of juvenile mosquitofish per treatment within the Pupfish experiment.

4.5. Discussion

The habitats were sufficient to support successful reproduction of poolfish when allopatric; however, poolfish produced virtually no juveniles in the presence of either population of mosquitofish. Thus, our findings are consistent with earlier work using a different source of mosquitofish (Chapter 2), further confirming the stated concern of exotic fishes as a threat to poolfish recovery (USFWS 1980). Our finding show that differences in mosquitofish body size were did not make a difference, and the mere presence of mosquitofish is likely to have important impacts on poolfish. These findings support the hypothesis that the invasion of mosquitofish likely contributed to the extirpation of the Corn Creek poolfish population in the mid 1970's (Selby 1977; see Chapter 1).

The responses of poolfish to mosquitofish were fundamentally different from the responses of pupfish. Although the numbers of juvenile pupfish were reduced in sympatry, there was still substantial juvenile pupfish production in the sympatric tanks with 100-200 pupfish juveniles per mesocosm. Although not significant, there was a trend toward higher production of juveniles when sympatric with Garrett compared to pupfish juvenile production when sympatric with Wabuska. This finding was contrary to our predictions that the larger Garrett phenotype with a larger gape size would consume more larvae. Thus, it appears that source population may be important in mediating impacts of mosquitofish on pupfish, but the mechanisms of these differential impacts need to be further explored.

One possible explanation for our findings is an apparent behavioral difference between the two mosquitofish populations. We observed Wabuska mosquitofish tended to be more benthic than Garrett, and were more skittish during feeding. Sumpter (2006) suggests groups of animals show collective behavior (e.g. aggressiveness, foraging, etc.) that may be different from individuals, and each individual of a group has different behavioral intricacies that contribute to the group's behavior. Differences in group dynamics between the native habitats to the mesocosm may have played a role in the interactions. Another possible explanation for the higher impacts of the smaller-bodied Wabuska mosquitofish could be due to unexpected compensatory growth of mosquitofish adults when placed in our common garden environment where they were provided an abundance of food; 5% of collective starting collective fish biomass. In fact, the biomass of adult Wabuska mosquitofish increased by 72% when in allopatry, but by 118% when sympatric with pupfish. These findings are consistent with earlier work showing evidence of countergradient variation for fat reserves. Wild Wabuska fish had relatively low fat reserves (6 – 7 %), whereas F-2 Wabuska fish raised under benign conditions had relatively high fat reserves (~26%; Stockwell and Weeks 1999; Stockwell and Vinyard 2000).

Even though body size of the adults rapidly increased for the Wabuska population, at the conclusion of the experiment Garrett adults were still larger and had a larger gape sizes (U=1862.500, P<0.001)(4.24 mm ± 0.05) than Wabuska adults (3.87mm ± 0.05). Thus, our findings lead us to reject the hypothesis that impacts of mosquitofish are mediated by gape-size limitation. We instead argue that other population specific differences in metabolism and/or growth rates may drive the higher impacts of Wabuska mosquitofish on pupfish juvenile production.

In conclusion, we saw repeatability of impacts of mosquitofish on poolfish and pupfish as reported in chapter 3. In general, poolfish populations were not sustainable in the presence of any of the mosquitofish populations used across both experiments. By contrast, pupfish apparently can successfully produce juveniles in the presence of mosquitofish. Our work is consistent with field observations showing co-persistence of pupfish with mosquitofish, and the limited observations where invasive species were associated with extirpations of poolfish populations. Our findings also suggest that mosquitofish impacts may vary depending on the particular population of mosquitofish, but additional work is necessary to further explore this hypothesis.

4.6. Acknowledgements

I thank S. Kainz, H. Hundt, J. Fisher, K. McLean, M. Stockwell and H. Fairfield for assistance with field work; S. Parmenter (California Department of Fish and Game), J. Harter, L. Simons (US Fish and Wildlife Service), J Sjoberg and K. Guadalupe, (Nevada Department of Wildlife) and R. Keller (Nevada Division of State Parks) for logistical and field support. This work has been conducted under USFWS permit TE126141-2, Nevada Scientific Collecting Permit S 34628 and NDSU IACUC #12027. This work was supported by a Nevada State Wildlife Grant administered by J. Sjoberg to C.A.S. and S.G., as well as stipend support for S.G. from the NDSU Environmental & Conservation Sciences Graduate Program.

4.7. References

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.

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

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.

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.

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

Miller, R. R. 1961. Man and the changing fish fauna of the American Southwest. Papers of the Michigan Academy of Science, Arts and Letters 46: 365–404.

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

Palkovacs, E. P., M. C. Marshall, B. A. Lamphere, B. R. Lynch, D. J. Weese, D. F. Fraser, D. N. Reznick, C. M. Pringle, and M. 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(1523): 1617-1628.

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

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

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

Stockwell, C. A. and S. C. Weeks. 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 behaviour. Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1465): 5-22.

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

CHAPTER 5. SYNTHESIS

5.1. Consequences of Change and Interspecific Interactions

Conservation of imperiled species using *ex situ* refuges has become an important tool for the management of many aquatic species (Pister 1993; Minckley 1995; Ostermann et. al. 2001; Deacon and Williams 2011); however, there are several problems related to this that are addressed in this dissertation. First, refuges are typically established as a hedge against extinction and to maintain taxa for the use of restocking native habitat if the native population is extirpated. However, placing taxa in novel environments, such as refuges, may have unintended consequences such as phenotypic divergence of the refuge population from the source population. Such phenotypic and/or evolutionary change may result in refuge populations that are mismatched (maladapted) to their native habitat, making the *ex situ* population unsuitable for reintroduction. Second, suitable habitats are rare, and there are not enough to maintain all species in need of *ex situ* conservation in single species refuges. In some cases, prospective refuges populations may harbor invasive species *Gambusia affinis*) (mosquitofish) (e.g. Meffe 1985; Marsh and Langhorst 1988; Lowe 2000) or other fish species of conservation concern (Henkanaththegedara and Stockwell 2014, Henkanaththegedara et al. 2015). Thus, understanding how protected species respond to unique fish communities may be useful for assessing if multi-species refuges are a viable management option. I examined these questions focusing on the Pahrump poolfish.

With approximately 5 decades of experience with *ex situ* refuges of desert fish in the Southwestern United States (Miller 1961; Minckley 1995), many of these problems are only recently being realized (Stockwell et al, 2003, Wilcox and Martin 2006, Collyer et al, 2011;

Henkanaththegedara and Stockwell 2014). We have historically considered native fishes to be evolutionarily naïve and thus very vulnerable to non-native competitors and predators (Cox and Lima, 2006), but recent work has suggested that intraguild predation allowing co-persistence of desirable and undesired species (Henkanaththegedara and Stockwell, 2014). These findings have proven that *ex situ* conservation of desert fishes is not clear cut. Given the rarity of the fish and habitats, managers do not have the luxury to haphazardly move species, which may have a host of undesirable effects (Ricciardi and Simberloff 2009). As such with this dissertation, it is important to examine existing characteristics of translocated populations and experimentally test the effects of these *ex situ* habitats on the imperiled species, as well as to test if multi-species refuges can be used to conserve rare fishes.

Phenotypic divergence of newly established populations of fish has been well documented (Stockwell and Weeks, 1999; Reznick and Ghalambor, 2001; Stockwell et al., 2003; Hendry et al., 2000), and such rapid divergence can result in populations that are maladapted to the original native habitat (Reznick et al., 2004). Such phenotypic divergence has been demonstrated for refuge populations of the highly endangered Devils Hole pupfish (Wilcox and Martin 2006), as well as recently established populations of the White Sands pupfish (Collyer et al. 2005, 2011). However, aside from these two case studies little is known how other protected fish may respond to novel conditions in refuge habitats. To further explore these questions, I examined phenotypic variation within and among refuge populations of the endangered Pahrump poolfish (*Empetrichthys latos*). This species was extirpated of its native habitat in 1975 and now only occurs in a variety of *ex situ* environments. Given the common ancestry of the fish in the refuges, the time spent in these environments, and the variation in the habitat types; the Pahrump poolfish is an ideal candidate to examine phenotypic differences of life history traits between these *ex situ* habitats.

Chapter 2 demonstrated substantial differences in size, fecundity, and fat content between populations of poolfish collected from two sites. This has shown that poolfish have different phenotypes in different refuges, possibly based on fish density and habitat size. Whether these changes reflect phenotypic plasticity and/or contemporary evolution has not yet been determined. However, these findings further support the idea that conditions in *ex situ* habitats may have a profound effect on the phenotypic variation and perhaps the evolutionary trajectory of targeted taxa. My findings have several management implications. For example, poolfish from the Spring Mountain site have greater fecundity, thus may genetically swamp a location if mixed with other Shoshone poolfish. However, the high fecundity of the Spring Mountain population may make it a good choice for establishing new refuges if managers desire rapid colonization of a site. Alternatively, larger Spring Mountain fish may be too large to effectively compete in smaller habitats where the smaller Shoshone ponds fish would be at an advantage; thus, if only a small pond is available Shoshone fish would be a better choice.

Given the lack of available suitable habitat for *ex situ* refuges, not only would it be beneficial to put several imperiled species per refuge, but also it may be necessary to consider habitats that already contain undesired species, such as the western mosquitofish. Chapter 3 provides experimental evidence that Pahrump poolfish would likely not be able to co-persist with pupfish or mosquitofish. By contrast, pupfish were able to persist in the presence of both mosquitofish and poolfish. These results support the observational data that mosquitofish are detrimental to poolfish, and provide guidance to managers for procuring habitat for future poolfish refuges (i.e. they need to avoid or remove mosquitofish). More importantly, it shows that pupfish may persist in *ex situ* habitats containing mosquitofish, and provides experimental evidence that some imperiled species may be maintained in *ex situ* habitats even if noxious undesirable species are present. Given the results of Chapter 3, one could ask if the effects of a noxious species is relevant at a species level or if different phenotypes within a species makes a difference in their effects. Chapter 4 addresses the question if the effects of Western mosquitofish are deterministic or based on phenotypes. We used large and small body size phenotypes of mosquitofish in mesocosms with pupfish and poolfish. Similar to Chapter 3, poolfish had essentially no surviving juveniles in either sympatric treatment. In addition, pupfish had reduced juvenile production when sympatric with either stock of mosquitofish, but nevertheless still experienced 10-20 times increase in population sizes. Oddly, pupfish juvenile production was higher for populations that were sympatric with large bodied mosquitofish compared to pupfish populations sympatric with smaller bodied mosquitofish. We observed differences in behavior and experienced compensatory growth, suggesting there were several unaccountable variables at work in our mesocosms.

In summary, I demonstrated life history variation exists among *ex situ* refuges of Pahrump poolfish, a finding that has implications for establishing new refuges of this endangered fish species. I also found that poolfish and pupfish have different responses to the presence of other species leading me to conclude that Pahrump poolfish must be managed in simple systems devoid of fish predators or competitors. By contrast, it is promising that species such as pupfish may be managed in multi-species refuges. Thus *ex situ* refuges may provide a canvas to maintain these species in a world that increasingly changing in the presence of habitat destruction, alien species and climate change.

5.2. References

Collyer M. L., J. S. Heilveil, C. A. Stockwell. 2011. Contemporary Evolutionary Divergence for a Protected Species following Assisted Colonization. PLOS ONE 6(8), e22310.

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., 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.

Hendry, A.P., J. K. Wenburg, P. Bentzen,, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290(5491): 516-518.

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.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2000. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp.

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

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

Miller, R.R. 1961. Man and the changing fish fauna of the American Southwest. Papers of the Michigan Academy of Science, Arts and Letters 46: 365–404.

Minckley, W. L. 1995. Translocation as a Tool for Conserving Imperiled Fishes: Experiences in Western United States. Biological Conservation 72: 297-309.

Ostermann, S. D., J. R. Deforge, and W. D. Edge. 2001. Captive breeding and reintroduction evaluation criteria: a case study of peninsular bighorn sheep. Conservation Biology 15: 749–760.

Pister, E.P. 1993. Species in a bucket. Natural History 102: 14.

Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112: 183-198.

Reznick, D. N., L. Nunney, and H. Rodd. 2004. Empirical evidence for rapid evolution. Pp 101-118. *In* Evolutionary Conservation Biology. D. Couvet, U. Diekman, and R. Ferrier. (eds.). Cambridge University Press.

Ricciardi A. and D. Simberloff. 2009. Assisted colonization is not a viable conservation strategy. Trends in Ecology and Evolution 24: 248–253.

Stockwell, C. A., A. P. Hendry, and M.T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18: 94–101

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.

Wilcox, J. L. and Martin, A. P. 2006. The devil's in the details: Genetic and phenotypic divergence between artificial and native populations of the endangered pupfish (Cyprinodon diabolis). Animal Conservation 9: 316-321.

APPENDIX. LENGTH, WEIGHT, AND GENDERS OF PAHRUMP POOLFISH (*EMPETRICHTHYS LATOS*) COLLECTED FROM SPRING MOUNTAIN RANCH (LAKE HARRIET), SHOSHONE PONDS, AND SHOSHONE STREAM

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	ssp-5-13	F	40.5	47.2	
Shoshone Stock Pond	ssp-5-13	F	36.5	42.2	1.39
Shoshone Stock Pond	ssp-5-13	M	34.6	40.5	1.32
Shoshone Stock Pond	ssp-5-13	F	38.1	44.4	1.64
Shoshone Stock Pond	ssp-5-13	F	39.9	46.6	1.87
Shoshone Stock Pond	ssp-5-13	F	37.7	43.7	1.54
Shoshone Stock Pond	ssp-5-13	F	33.7	40.9	1.20
Shoshone Stock Pond	ssp-5-13	F	47.0	52.5	2.49
Shoshone Stock Pond	ssp-5-13	F	35.2	41.3	1.30
Shoshone Stock Pond	ssp-5-13	F	38.8	45.5	1.65
Shoshone Stock Pond	ssp-5-13	F	37.0	42.9	1.40
Shoshone Stock Pond	ssp-5-13	F	43.7	50.3	2.65
Shoshone Stock Pond	ssp-5-13	F	40.6	47.8	2.12
Shoshone Stock Pond	ssp-5-13	M	31.9	37.8	0.92
Shoshone Stock Pond	ssp-5-13	M	33.9	40.3	1.12
Shoshone Stock Pond	ssp-5-13	F	34.2	39.9	1.11
Shoshone Stock Pond	ssp-5-13	F	37.2	44.6	1.54
Shoshone Stock Pond	ssp-5-13	F	37.3	42.7	1.39
Shoshone Stock Pond	ssp-5-13	F	34.5	38.6	1.12
Shoshone Stock Pond	ssp-5-13	F	34.7	41.0	1.28
Shoshone Stock Pond	ssp-5-13	F	37.2	42.5	1.45
Shoshone Stock Pond	ssp-5-13	F	40.0	46.0	1.70
Shoshone Stock Pond	ssp-5-13	F	32.4	38.7	
Shoshone Stock Pond	ssp-5-13	F	34.6	41.4	1.23
Shoshone Stock Pond	ssp-5-13	F	38.2	43.3	1.61
Shoshone Stock Pond	ssp-5-13	F	37.6	42.2	1.36
Shoshone Stock Pond	ssp-5-13	F	37.1	42.8	1.33
Shoshone Stock Pond	SSP-5-14	M	40.2	45.8	1.80
Shoshone Stock Pond	SSP-5-14	F	40.7	48.4	1.82

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	SSP-5-14	F	53.2	59.5	4.15
Shoshone Stock Pond	SSP-5-14	F	45.0	52.9	2.50
Shoshone Stock Pond	SSP-5-14	M	34.9	39.8	1.04
Shoshone Stock Pond	SSP-5-14	M	35.4	42.4	1.29
Shoshone Stock Pond	SSP-5-14	F	41.7	46.9	1.76
Shoshone Stock Pond	SSP-5-14	M	36.3	40.8	1.30
Shoshone Stock Pond	SSP-5-14	F	43.5	49.9	2.25
Shoshone Stock Pond	SSP-5-14	F	44.8	50.4	2.20
Shoshone Stock Pond	SSP-5-14	F	43.4	50.7	2.27
Shoshone Stock Pond	SSP-5-14	F	43.6	50.8	
Shoshone Stock Pond	SSP-5-14	F	42.7	48.7	1.94
Shoshone Stock Pond	SSP-5-14	M	34.3	39.4	1.10
Shoshone Stock Pond	SSP-5-14	F	39.9	45.6	1.70
Shoshone Stock Pond	SSP-5-14	M	31.8	38.9	0.91
Shoshone Stock Pond	SSP-5-14	F	39.6	44.8	
Shoshone Stock Pond	SSP-5-14	F	36.4	42.8	1.41
Shoshone Stock Pond	SSP-5-14	F	40.1	47.0	1.83
Shoshone Stock Pond	SSP-5-14	M	35.8	41.0	1.39
Shoshone Stock Pond	SSP-5-14	F	46.6	53.8	2.74
Shoshone Stock Pond	SSP-5-14	F	47.7	53.4	2.81
Shoshone Stock Pond	SSP-5-14	M	33.7	39.7	1.16
Shoshone Stock Pond	SSP-5-14	F	37.0	42.0	1.33
Shoshone Stock Pond	SSP-5-14	F	37.7	43.6	1.36
Shoshone Stock Pond	SSP-5-14	M	41.9	47.2	2.05
Shoshone Stock Pond	SSP-5-14	F	39.8	46.5	1.65
Shoshone Stock Pond	SSP-5-14	F	45.1	52.6	2.41
Shoshone Stock Pond	SSP-5-14	F	46.6	53.6	2.51
Shoshone Stock Pond	SSP-5-14	F	38.3	44.6	1.65
Shoshone Stock Pond	SSP-5-14	F	43.1	49.4	2.07
Shoshone Stock Pond	SSP-5-14	F	50.1	55.3	2.79
Shoshone Stock Pond	SSP-5-14	F	38.5	45.6	1.80
Shoshone Stock Pond	SSP-5-14	F	44.4	51.6	2.32
Shoshone Stock Pond	SSP-5-14	M	36.8	42.0	1.32
Shoshone Stock Pond	SSP-5-14	F	43.7	49.6	2.33
Shoshone Stock Pond	SSP-5-14	F	47.0	54.1	2.87
Shoshone Stock Pond	SSP-5-14	F	47.6	55.6	3.04

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	SSP-5-14	F	41.4	48.2	1.92
Shoshone Stock Pond	SSP-5-14	F	44.1	51.3	2.42
Shoshone Stock Pond	SSP-5-14	F	40.7	47.8	2.08
Shoshone Stock Pond	SSP-5-14	F	37.4	43.8	1.50
Shoshone Stock Pond	SSP-5-14	F	39.2	44.6	1.70
Shoshone Stock Pond	SSP-5-14	F	40.9	47.4	1.95
Shoshone Stock Pond	SSP-5-14	M	38.8	44.4	1.73
Shoshone Stock Pond	SSP-5-14	F	42.8	48.9	2.12
Shoshone Stock Pond	SSP-5-14	F	40.8	44.4	1.55
Shoshone Stock Pond	SSP-5-14	M	34.3	39.5	
Shoshone Stock Pond	SSP-5-14	F	39.2	45.5	1.57
Shoshone Stock Pond	SSP-6-14	F	45.7	52.1	2.40
Shoshone Stock Pond	SSP-6-14	F	50.0	56.7	3.17
Shoshone Stock Pond	SSP-6-14	F	43.0	48.7	2.02
Shoshone Stock Pond	SSP-6-14	F	45.0	52.9	2.59
Shoshone Stock Pond	SSP-6-14	F	39.8	45.9	1.63
Shoshone Stock Pond	SSP-6-14	F	45.3	51.0	2.39
Shoshone Stock Pond	SSP-6-14	F	40.9	45.5	1.64
Shoshone Stock Pond	SSP-6-14	F	49.5	56.3	3.25
Shoshone Stock Pond	SSP-6-14	F	51.2	56.8	3.69
Shoshone Stock Pond	SSP-6-14	F	45.7	52.4	2.46
Shoshone Stock Pond	SSP-6-14	F	46.0	52.5	2.67
Shoshone Stock Pond	SSP-6-14	F	49.6	55.0	3.21
Shoshone Stock Pond	SSP-6-14	F	44.8	49.8	2.09
Shoshone Stock Pond	SSP-6-14	F	40.6	46.2	1.61
Shoshone Stock Pond	SSP-6-14	F	47.6	55.4	3.03
Shoshone Stock Pond	SSP-6-14	F	50.5	58.1	3.44
Shoshone Stock Pond	SSP-6-14	F	50.8	58.1	3.32
Shoshone Stock Pond	SSP-6-14	F	37.3	42.4	1.32
Shoshone Stock Pond	SSP-6-14	M	33.3	39.0	0.96
Shoshone Stock Pond	SSP-6-14	F	39.5	45.4	1.58
Shoshone Stock Pond	SSP-6-14	F	46.1	53.0	2.35
Shoshone Stock Pond	SSP-6-14	F	30.5	36.4	0.81
Shoshone Stock Pond	SSP-6-14	F	44.6	51.5	2.53
Shoshone Stock Pond	SSP-6-14	M	33.3	38.7	0.99
Shoshone Stock Pond	SSP-6-14	F	38.9	47.3	1.60

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	SSP-6-14	F	40.6	47.2	1.87
Shoshone Stock Pond	SSP-6-14	F	39.6	45.3	1.48
Shoshone Stock Pond	SSP-6-14	M	31.5	37.5	0.86
Shoshone Stock Pond	SSP-6-14	F	45.9	52.8	
Shoshone Stock Pond	SSP-6-14	F	40.1	46.2	1.80
Shoshone Stock Pond	SSP-6-14	F	41.2	47.2	1.74
Shoshone Stock Pond	SSP-6-14	F	49.7	58.2	3.08
Shoshone Stock Pond	SSP-6-14	F	42.2	48.9	2.03
Shoshone Stock Pond	SSP-6-14	F	41.2	46.5	1.97
Shoshone Stock Pond	SSP-6-14	F	43.3	50.0	2.15
Shoshone Stock Pond	SSP-6-14	M	33.5	38.0	1.09
Shoshone Stock Pond	SSP-6-14	F	48.5	55.4	3.08
Shoshone Stock Pond	SSP-6-14	F	43.3	49.2	2.09
Shoshone Stock Pond	SSP-6-14	F	48.1	54.8	3.13
Shoshone Stock Pond	SSP-6-14	F	41.3	47.4	1.79
Shoshone Stock Pond	SSP-6-14	F	49.3	57.2	2.99
Shoshone Stock Pond	SSP-6-14	F	40.1	45.3	1.74
Shoshone Stock Pond	SSP-6-14	M	30.0	35.2	0.75
Shoshone Stock Pond	SSP-6-14	M	36.6	43.2	1.26
Shoshone Stock Pond	SSP-6-14	F	43.2	49.6	2.16
Shoshone Stock Pond	SSP-6-14	F	40.1	46.6	1.84
Shoshone Stock Pond	SSP-6-14	F	38.8	46.0	1.63
Shoshone Stock Pond	SSP-6-14	F	38.0	45.0	1.50
Shoshone Stock Pond	SSP-6-14	F	37.5	44.1	1.53
Shoshone Stock Pond	SSP-6-14	M	29.7	34.3	0.66
Shoshone Stock Pond	SSP-7-14	F	48.2	54.5	2.83
Shoshone Stock Pond	SSP-7-14	F	44.8	51.9	2.74
Shoshone Stock Pond	SSP-7-14	F	41.9	48.2	2.03
Shoshone Stock Pond	SSP-7-14	F	41.1	48.6	2.41
Shoshone Stock Pond	SSP-7-14	F	39.5	46.4	1.90
Shoshone Stock Pond	SSP-7-14	M	33.0	39.1	1.14
Shoshone Stock Pond	SSP-7-14	F	44.2	50.0	2.27
Shoshone Stock Pond	SSP-7-14	F	41.6	49.6	2.36
Shoshone Stock Pond	SSP-7-14	M	31.4	38.1	1.01
Shoshone Stock Pond	SSP-7-14	F	38.1	44.0	1.55
Shoshone Stock Pond	SSP-7-14	F	43.5	50.5	2.42

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	SSP-7-14	F	40.7	46.7	1.86
Shoshone Stock Pond	SSP-7-14	F	41.3	48.8	2.12
Shoshone Stock Pond	SSP-7-14	F	41.9	49.0	2.00
Shoshone Stock Pond	SSP-7-14	F	39.1	45.7	1.71
Shoshone Stock Pond	SSP-7-14	M	32.6	37.7	0.99
Shoshone Stock Pond	SSP-7-14	F	40.8	47.0	1.81
Shoshone Stock Pond	SSP-7-14	F	50.7	57.4	3.62
Shoshone Stock Pond	SSP-7-14	F	33.5	38.7	1.02
Shoshone Stock Pond	SSP-7-14	F	48.4	56.3	3.31
Shoshone Stock Pond	SSP-7-14	F	50.0	59.9	4.35
Shoshone Stock Pond	SSP-7-14	F	44.9	54.0	2.97
Shoshone Stock Pond	SSP-7-14	F	47.5	55.8	3.35
Shoshone Stock Pond	SSP-7-14	F	31.4	36.8	0.84
Shoshone Stock Pond	SSP-7-14	F	39.7	46.7	1.83
Shoshone Stock Pond	SSP-7-14	F	41.3	51.1	2.18
Shoshone Stock Pond	SSP-7-14	F	48.9	58.3	3.58
Shoshone Stock Pond	SSP-7-14	F	44.5	53.1	2.78
Shoshone Stock Pond	SSP-7-14	F	43.2	49.7	2.09
Shoshone Stock Pond	SSP-7-14	F	36.5	45.8	1.69
Shoshone Stock Pond	SSP-7-14	F	44.6	52.4	2.45
Shoshone Stock Pond	SSP-7-14	F	37.5	44.5	1.73
Shoshone Stock Pond	SSP-7-14	F	44.3	53.3	2.74
Shoshone Stock Pond	SSP-7-14	F	34.2	40.4	1.05
Shoshone Stock Pond	SSP-7-14	M	29.7	35.4	0.74
Shoshone Stock Pond	SSP-7-14	F	43.3	51.5	2.85
Shoshone Stock Pond	SSP-7-14	F	38.7	46.2	1.84
Shoshone Stock Pond	SSP-7-14	F	52.3	60.4	3.94
Shoshone Stock Pond	SSP-7-14	M	33.5	40.4	1.16
Shoshone Stock Pond	SSP-7-14	F	41.5	48.3	2.16
Shoshone Stock Pond	SSP-7-14	F	45.8	54.1	2.93
Shoshone Stock Pond	SSP-7-14	F	44.5	52.8	3.02
Shoshone Stock Pond	SSP-7-14	F	46.8	54.4	2.97
Shoshone Stock Pond	SSP-7-14	M	32.5	39.4	1.05
Shoshone Stock Pond	SSP-7-14	F	40.4	47.9	2.32
Shoshone Stock Pond	SSP-7-14	F	31.8	38.9	0.95
Shoshone Stock Pond	SSP-7-14	F	31.2	37.8	0.87

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stock Pond	SSP-7-14	F	47.6	55.5	3.43
Shoshone Stock Pond	SSP-7-14	F	42.0	49.1	2.20
Shoshone Stock Pond	SSP-7-14	F	42.0	48.9	2.17
Shoshone Stock Pond	SSP-7-14	F	31.6	36.4	0.82
Shoshone Stock Pond	SSP-7-14	M	30.7	37.1	0.76
Shoshone Stock Pond	SSP-7-14	F	43.0	51.2	2.30
Shoshone Stock Pond	SSP-7-14	F	45.2	52.2	2.36
Shoshone Stock Pond	SSP-7-14	F	52.9	61.4	4.76
Shoshone Stock Pond	SSP-7-14	F	52.3	61.7	4.35
Shoshone Stock Pond	SSP-7-14	F	41.9	49.7	2.38
Shoshone Stock Pond	SSP-7-14	F	41.9	48.8	1.99
Shoshone Stock Pond	SSP-7-14	F	42.4	50.5	2.30
Shoshone Stock Pond	SSP-7-14	F	42.2	50.1	2.44
Shoshone Stock Pond	SSP-7-14	M	29.4	36.0	0.83
Shoshone Stock Pond	SSP-7-14	F	30.2	35.6	0.79
Shoshone Stock Pond	SSP-7-14	F	31.8	37.8	0.87
Shoshone Stock Pond	SSP-7-14	F	41.7	48.8	2.23
Shoshone Stock Pond	SSP-7-14	M	30.0	36.2	0.88
Shoshone Stock Pond	SSP-7-14	F	34.6	40.7	1.12
Shoshone Stock Pond	SSP-7-14	M	36.0	42.9	1.39
Shoshone Stock Pond	SSP-7-14	M	36.6	43.6	1.44
Shoshone Stock Pond	SSP-7-14	M	27.6	32.5	0.57
Shoshone Stock Pond	SSP-7-14	F	34.9	41.7	1.22
Shoshone Stock Pond	SSP-7-14	F	30.1	36.1	0.77
Shoshone Stock Pond	SSP-7-14	F	34.1	39.7	1.11
Shoshone Stock Pond	SSP-7-14	M	29.3	34.8	0.77
Shoshone Stream	sst-5-13	F	51.8	58.0	3.40
Shoshone Stream	sst-5-13	F	37.6	43.6	1.50
Shoshone Stream	sst-5-13	F	36.8	41.5	1.31
Shoshone Stream	sst-5-13	M	40.9	46.3	1.90
Shoshone Stream	sst-5-13	F	42.1	47.4	2.06
Shoshone Stream	sst-5-13	F	51.4	57.4	3.51
Shoshone Stream	sst-5-13	F	47.1	53.8	2.78
Shoshone Stream	sst-5-13	M	34.0	34.7	0.70
Shoshone Stream	sst-5-13	M	36.1	39.9	1.24
Shoshone Stream	sst-5-13	M	33.5	36.5	0.87

Site	**ID**	**Gender**	**SL**	**TL**	**Wet Mass**
Shoshone Stream	sst-5-13	F	38.4	42.5	1.37
Shoshone Stream	sst-5-13	M	42.6	46.5	1.80
Shoshone Stream	sst-5-13	F	42.0	46.5	1.70
Shoshone Stream	sst-5-13	F	35.9	41.9	1.22
Shoshone Stream	sst-5-13	F	36.3	42.8	1.34
Shoshone Stream	sst-5-13	M			0.85
Shoshone Stream	sst-5-13	F	39.3	44.1	1.58
Shoshone Stream	sst-5-13	M	32.8	38.7	0.99
Shoshone Stream	sst-5-13	F	38.1	43.0	1.56
Shoshone Stream	sst-5-13	F	41.9	47.3	1.75
Shoshone Stream	sst-5-13	F	46.2	52.1	2.42
Shoshone Stream	sst-5-13	M	28.9	32.8	0.62
Shoshone Stream	sst-5-13	M	34.7	39.5	1.06
Shoshone Stream	sst-5-13	M	31.2	36.3	0.80
Shoshone Stream	sst-5-13	F	38.0	43.5	1.31
Shoshone Stream	SST-7-14	F	66.2	58.1	5.46
Shoshone Stream	SST-7-14	F	39.1	43.0	1.16
Shoshone Stream	SST-7-14	M	32.1	36.0	0.76
Shoshone Stream	SST-7-14	M	38.3	41.2	1.22
Shoshone Stream	SST-7-14	M	39.1	45.0	1.66
Shoshone Stream	SST-7-14	F	37.6	41.3	1.22
Shoshone Stream	SST-7-14	M	32.1	35.3	0.76
Shoshone Stream	SST-7-14	M	36.4	39.0	1.10
Shoshone Stream	SST-7-14	M	30.7	34.7	0.68
Shoshone Stream	SST-7-14	M	31.7	35.2	0.69
Shoshone Stream	SST-7-14	M	32.4	36.5	0.77
Shoshone Stream	SST-7-14	F	36.4	40.5	1.07
Shoshone Stream	SST-7-14	M	23.8	26.8	0.32
Shoshone Stream	SST-7-14	M	23.4	25.8	0.31
Shoshone Stream	SST-7-14	M	37.3	41.6	1.06
Shoshone Stream	SST-7-14	M	29.3	32.8	0.65
Shoshone Stream	SST-7-14	M	38.1	41.5	1.21
Shoshone Stream	SST-7-14	M	38.4	43.5	1.26
Shoshone Stream	SST-7-14	M	29.7	33.5	0.62
Shoshone Stream	SST-7-14	F	32.0	35.5	0.75
Shoshone Stream	SST-7-14	M	34.9	39.3	1.03

Site	ID	Gender	SL	TL	Wet Mass
Shoshone Stream	SST-7-14	M	35.7	39.9	1.05
Shoshone Stream	SST-7-14	M	18.6	21.1	0.16
Shoshone Stream	SST-7-14	M	26.7	29.0	0.38
Shoshone Stream	SST-7-14	F	33.5	36.0	0.82
Shoshone Stream	SST-7-14	M	23.5	25.9	0.31
Shoshone Stream	SST-7-14	M	22.4	24.9	0.25
Shoshone Stream	SST-7-14	M	17.3	19.9	0.12
Shoshone Stream	SST-7-14	M	24.9	28.2	0.39
Shoshone Stream	SST-7-14	M	30.0	32.4	0.62
Shoshone Stream	SST-7-14	M	26.2	29.5	0.42
Shoshone Stream	SST-7-14	M	28.8	32.0	0.62
Shoshone Stream	SST-7-14	M	27.0	30.1	0.45
Shoshone Stream	SST-7-14	M	29.1	33.0	0.65
Shoshone Stream	SST-7-14	M	29.4	32.3	0.59
Shoshone Stream	SST-7-14	M	27.8	32.5	0.62
Shoshone Stream	SST-7-14	M	17.1		0.11
Spring Mountain Ranch	smr-5-13	F	49.9	56.6	3.51
Spring Mountain Ranch	smr-5-13	F	47.8	55.3	3.17
Spring Mountain Ranch	smr-5-13	M	34.2	40.3	1.02
Spring Mountain Ranch	smr-5-13	F	39.3	48.0	1.73
Spring Mountain Ranch	smr-5-13	F	56.6	64.8	5.14
Spring Mountain Ranch	smr-5-13	F	48.6	57.0	3.41
Spring Mountain Ranch	smr-5-13	M	30.9	37.0	0.82
Spring Mountain Ranch	smr-5-13	F	46.2	52.9	2.59
Spring Mountain Ranch	smr-5-13	M	37.6	43.0	1.39
Spring Mountain Ranch	smr-5-13	M	34.0	41.0	1.09
Spring Mountain Ranch	smr-5-13	F	58.8	63.9	5.29
Spring Mountain Ranch	smr-5-13	F	50.6	60.0	3.75
Spring Mountain Ranch	smr-5-13	F	50.5	57.9	3.53
Spring Mountain Ranch	smr-5-13	M	34.3	40.4	1.08
Spring Mountain Ranch	smr-5-13	M	32.1	36.8	0.77
Spring Mountain Ranch	smr-5-13	M	36.3	41.4	1.01
Spring Mountain Ranch	smr-5-13	M	31.0	37.4	0.76
Spring Mountain Ranch	smr-5-13	M			1.33
Spring Mountain Ranch	smr-5-13	M	37.3	43.6	1.37
Spring Mountain Ranch	smr-5-13	M	30.3	35.5	0.58

Site	ID	Gender	SL	TL	Wet Mass
Spring Mountain Ranch	smr-5-13	M	37.3	45.6	1.63
Spring Mountain Ranch	smr-5-13	M	32.6	39.1	0.97
Spring Mountain Ranch	smr-5-13	M	37.9	42.9	1.34
Spring Mountain Ranch	smr-5-13	M	30.6	36.2	0.82
Spring Mountain Ranch	smr-5-13	F	66.4	73.7	7.95
Spring Mountain Ranch	smr-5-13	M	37.5	43.2	1.53
Spring Mountain Ranch	smr-5-13	F	66.9	75.8	8.30
Spring Mountain Ranch	SMR-5-14	M	34.3	40.4	1.13
Spring Mountain Ranch	SMR-5-14	M	38.0	45.2	1.58
Spring Mountain Ranch	SMR-5-14	F	51.6	57.2	3.59
Spring Mountain Ranch	SMR-5-14	F	47.1	52.3	2.47
Spring Mountain Ranch	SMR-5-14	F	44.2	50.0	2.33
Spring Mountain Ranch	SMR-5-14	F	60.4	68.2	6.06
Spring Mountain Ranch	SMR-5-14	M	42.0	48.6	2.18
Spring Mountain Ranch	SMR-5-14	F	45.0	52.7	2.38
Spring Mountain Ranch	SMR-5-14	F	49.9	56.9	3.36
Spring Mountain Ranch	SMR-5-14	F	46.6	51.4	2.43
Spring Mountain Ranch	SMR-5-14	F	41.0	46.8	1.67
Spring Mountain Ranch	SMR-5-14	M	36.1	42.1	1.31
Spring Mountain Ranch	SMR-5-14	M	40.4	45.0	1.91
Spring Mountain Ranch	SMR-5-14	M	38.6	43.5	1.28
Spring Mountain Ranch	SMR-5-14	M	35.0	40.2	1.08
Spring Mountain Ranch	SMR-5-14	M	39.4	44.8	1.62
Spring Mountain Ranch	SMR-5-14	M	34.2	39.3	0.97
Spring Mountain Ranch	SMR-5-14	M	39.2	44.8	1.49
Spring Mountain Ranch	SMR-5-14	F	56.3	64.5	5.44
Spring Mountain Ranch	SMR-5-14	F	45.0	50.3	2.54
Spring Mountain Ranch	SMR-5-14	F	57.8	63.8	5.20
Spring Mountain Ranch	SMR-5-14	F	48.5	55.7	2.94
Spring Mountain Ranch	SMR-5-14	M	38.4	45.0	1.64
Spring Mountain Ranch	SMR-5-14	M	36.0	41.1	1.15
Spring Mountain Ranch	SMR-5-14	M	32.7	38.4	0.92
Spring Mountain Ranch	SMR-5-14	M	39.3	45.3	1.83
Spring Mountain Ranch	SMR-5-14	M	36.2	43.0	1.32
Spring Mountain Ranch	SMR-5-14	M	38.2	44.9	1.55
Spring Mountain Ranch	SMR-5-14	F	56.0	64.6	5.05

Site	ID	Gender	SL	TL	Wet Mass
Spring Mountain Ranch	SMR-5-14	M	36.9	41.3	1.31
Spring Mountain Ranch	SMR-5-14	M	38.4	44.0	1.42
Spring Mountain Ranch	SMR-5-14	M	43.5	48.7	2.12
Spring Mountain Ranch	SMR-5-14	F	59.3	65.7	5.20
Spring Mountain Ranch	SMR-5-14	M	37.2	43.9	1.39
Spring Mountain Ranch	SMR-5-14	M	38.7	45.7	1.63
Spring Mountain Ranch	SMR-5-14	M	36.9	42.9	1.42
Spring Mountain Ranch	SMR-5-14	M	40.5	45.5	1.65
Spring Mountain Ranch	SMR-5-14	M	34.6	39.2	1.14
Spring Mountain Ranch	SMR-5-14	F	48.8	55.3	3.20
Spring Mountain Ranch	SMR-5-14	M	39.9	46.8	1.82
Spring Mountain Ranch	SMR-5-14	F	63.0	70.6	6.45
Spring Mountain Ranch	SMR-5-14	M	43.1	49.4	2.07
Spring Mountain Ranch	SMR-5-14	F	46.5	53.9	2.81
Spring Mountain Ranch	SMR-5-14	M	42.5	48.4	2.17
Spring Mountain Ranch	SMR-5-14	F	63.7	71.4	6.56
Spring Mountain Ranch	SMR-5-14	F	50.0	56.4	3.48
Spring Mountain Ranch	SMR-5-14	F	51.3	59.6	3.95
Spring Mountain Ranch	SMR-5-14	M	37.1	42.0	1.35
Spring Mountain Ranch	SMR-5-14	M	37.0	42.5	1.27
Spring Mountain Ranch	SMR-5-14	F	49.5	58.4	3.63
Spring Mountain Ranch	SMR-5-14	M	38.0	44.4	1.50
Spring Mountain Ranch	SMR-5-14	M	44.0	48.9	2.20
Spring Mountain Ranch	SMR-5-14	M	39.8	45.0	1.57
Spring Mountain Ranch	SMR-5-14	M	39.6	45.9	1.56
Spring Mountain Ranch	SMR-5-14	M	38.8	44.7	1.48
Spring Mountain Ranch	SMR-6-14	F	79.2	88.6	14.94
Spring Mountain Ranch	SMR-6-14	F	46.8	53.2	3.06
Spring Mountain Ranch	SMR-6-14	F	68.2	77.4	9.90
Spring Mountain Ranch	SMR-6-14	F	44.1	50.1	2.51
Spring Mountain Ranch	SMR-6-14	F	58.8	66.1	5.83
Spring Mountain Ranch	SMR-6-14	M	36.9	42.8	1.49
Spring Mountain Ranch	SMR-6-14	F	49.6	58.8	4.03
Spring Mountain Ranch	SMR-6-14	F	51.8	57.9	3.94
Spring Mountain Ranch	SMR-6-14	F	45.6	52.6	2.62
Spring Mountain Ranch	SMR-6-14	M	37.1	43.8	1.39

Site	ID	Gender	SL	TL	Wet Mass
Spring Mountain Ranch	SMR-6-14	F	57.0	64.8	5.75
Spring Mountain Ranch	SMR-6-14	F	53.4	60.2	3.82
Spring Mountain Ranch	SMR-6-14	F	75.1	83.1	10.67
Spring Mountain Ranch	SMR-6-14	F	57.4	65.5	5.81
Spring Mountain Ranch	SMR-6-14	F	65.7	73.9	8.01
Spring Mountain Ranch	SMR-6-14	F	53.2	60.2	4.44
Spring Mountain Ranch	SMR-6-14	F	66.5	74.7	8.73
Spring Mountain Ranch	SMR-6-14	F	50.6	57.1	4.13
Spring Mountain Ranch	SMR-6-14	F	50.5	56.1	3.16
Spring Mountain Ranch	SMR-6-14	F	67.1	74.5	8.50
Spring Mountain Ranch	SMR-6-14	F	50.1	57.2	3.86
Spring Mountain Ranch	SMR-6-14	F	57.6	64.5	5.47
Spring Mountain Ranch	SMR-6-14	F	43.8	49.8	2.44
Spring Mountain Ranch	SMR-6-14	F	56.1	63.4	5.35
Spring Mountain Ranch	SMR-6-14	F	57.2	65.7	5.47
Spring Mountain Ranch	SMR-6-14	F	69.7	77.5	9.51
Spring Mountain Ranch	SMR-6-14	F	50.1	56.7	3.38
Spring Mountain Ranch	SMR-6-14	F	47.5	55.0	3.19
Spring Mountain Ranch	SMR-6-14	F	60.0	69.4	6.48
Spring Mountain Ranch	SMR-6-14	M	35.6	40.8	1.21
Spring Mountain Ranch	SMR-6-14	F	49.4	56.1	3.42
Spring Mountain Ranch	SMR-6-14	F	53.1	61.6	4.74
Spring Mountain Ranch	SMR-6-14	F	57.3	64.2	5.72
Spring Mountain Ranch	SMR-6-14	F	57.0	64.8	6.34
Spring Mountain Ranch	SMR-6-14	F	54.0	61.9	
Spring Mountain Ranch	SMR-6-14	F	59.2	66.8	6.11
Spring Mountain Ranch	SMR-6-14	F	53.5	60.2	4.22
Spring Mountain Ranch	SMR-6-14	F	62.0	69.6	7.09
Spring Mountain Ranch	SMR-6-14	F	49.1	54.9	3.52
Spring Mountain Ranch	SMR-6-14	F	61.2	68.4	6.69
Spring Mountain Ranch	SMR-6-14	F	50.7	57.7	4.40
Spring Mountain Ranch	SMR-6-14	F	66.0	73.7	8.93
Spring Mountain Ranch	SMR-6-14	F	53.8	59.8	4.17
Spring Mountain Ranch	SMR-6-14	F	48.8	54.2	2.89
Spring Mountain Ranch	SMR-6-14	F	45.8	51.9	2.90
Spring Mountain Ranch	SMR-6-14	F	49.3	56.3	3.30

Site	ID	Gender	SL	TL	Wet Mass
Spring Mountain Ranch	SMR-6-14	F	51.7	59.0	4.15
Spring Mountain Ranch	SMR-6-14	F	52.2	58.3	3.79
Spring Mountain Ranch	SMR-6-14	F	54.6	63.6	4.67
Spring Mountain Ranch	SMR-6-14	M	36.0	39.9	1.12
Spring Mountain Ranch	SMR-8-14	F	51.1	59.7	4.15
Spring Mountain Ranch	SMR-8-14	F	46.3	54.7	
Spring Mountain Ranch	SMR-8-14	F	57.5	65.3	5.70
Spring Mountain Ranch	SMR-8-14	F	46.1	53.6	3.01
Spring Mountain Ranch	SMR-8-14	F	42.8	49.2	2.43
Spring Mountain Ranch	SMR-8-14	F	54.2	61.1	3.92
Spring Mountain Ranch	SMR-8-14	F	45.9	53.4	3.07
Spring Mountain Ranch	SMR-8-14	F	51.2	57.3	3.81
Spring Mountain Ranch	SMR-8-14	F	50.4	58.8	3.66
Spring Mountain Ranch	SMR-8-14	F	48.6	55.2	3.37
Spring Mountain Ranch	SMR-8-14	F	67.3	78.5	9.39
Spring Mountain Ranch	SMR-8-14	F	62.9	71.1	7.15
Spring Mountain Ranch	SMR-8-14	F	55.8	66.9	5.40
Spring Mountain Ranch	SMR-8-14	F	52.4	61.3	4.57
Spring Mountain Ranch	SMR-8-14	F	57.7	64.8	4.99
Spring Mountain Ranch	SMR-8-14	F	62.8	70.9	6.80
Spring Mountain Ranch	SMR-8-14	F	51.4	57.5	3.66
Spring Mountain Ranch	SMR-8-14	F	44.9	53.2	3.13
Spring Mountain Ranch	SMR-8-14	F	67.1	75.7	9.90
Spring Mountain Ranch	SMR-8-14	F	59.2	67.4	5.99
Spring Mountain Ranch	SMR-8-14	F	43.9	49.9	2.48
Spring Mountain Ranch	SMR-8-14	F	53.0	63.4	4.78
Spring Mountain Ranch	SMR-8-14	F	61.8	71.0	7.56
Spring Mountain Ranch	SMR-8-14	F	44.0	51.1	2.79
Spring Mountain Ranch	SMR-8-14	F	57.5	65.3	6.13
Spring Mountain Ranch	SMR-8-14	F	45.7	53.4	2.18
Spring Mountain Ranch	SMR-8-14	F	57.2	65.9	5.92
Spring Mountain Ranch	SMR-8-14	F	45.9	54.0	3.52
Spring Mountain Ranch	SMR-8-14	F	52.1	61.6	4.45
Spring Mountain Ranch	SMR-8-14	F	48.5	54.9	3.37
Spring Mountain Ranch	SMR-8-14	F	54.6	62.7	4.75
Spring Mountain Ranch	SMR-8-14	F	50.0	57.7	3.60

Site	ID	Gender	SL	TL	Wet Mass
Spring Mountain Ranch	SMR-8-14	F	54.2	60.6	4.43
Spring Mountain Ranch	SMR-8-14	F	55.6	61.7	5.15
Spring Mountain Ranch	SMR-8-14	F	61.4	70.8	6.69
Spring Mountain Ranch	SMR-8-14	F	51.0	58.8	4.09
Spring Mountain Ranch	SMR-8-14	F	46.6	54.7	3.14
Spring Mountain Ranch	SMR-8-14	F	48.5	56.0	3.35
Spring Mountain Ranch	SMR-8-14	F	53.5	61.1	4.71
Spring Mountain Ranch	SMR-8-14	F	53.6	61.0	4.31
Spring Mountain Ranch	SMR-8-14	F	49.6	55.9	4.00
Spring Mountain Ranch	SMR-8-14	M	36.3	42.0	1.19
Spring Mountain Ranch	SMR-8-14	F	55.0	63.5	4.70
Spring Mountain Ranch	SMR-8-14	F	53.0	62.9	4.77
Spring Mountain Ranch	SMR-8-14	F	54.7	61.5	4.43
Spring Mountain Ranch	SMR-8-14	F	53.5	61.4	4.46
Spring Mountain Ranch	SMR-8-14	F	51.1	58.9	3.71
Spring Mountain Ranch	SMR-8-14	F	47.9	57.5	3.36
Spring Mountain Ranch	SMR-8-14	F	53.5	60.8	4.33
Spring Mountain Ranch	SMR-8-14	F	48.9	57.2	3.40
Spring Mountain Ranch	SMR-8-14	F	51.6	59.6	4.24
Spring Mountain Ranch	SMR-8-14	F	57.5	66.9	5.68
Spring Mountain Ranch	SMR-8-14	M	29.9	35.4	0.92
Spring Mountain Ranch	SMR-8-14	F	46.1	53.8	3.17
Spring Mountain Ranch	SMR-8-14	F	44.7	53.2	3.06
Spring Mountain Ranch	SMR-8-14	F	56.9	64.4	5.60
Spring Mountain Ranch	SMR-8-14	F	63.3	70.4	8.45
Spring Mountain Ranch	SMR-8-14	F	55.4	65.2	5.22
Spring Mountain Ranch	SMR-8-14	F	55.1	63.2	4.81
Spring Mountain Ranch	SMR-8-14	F	50.7	58.7	3.81
Spring Mountain Ranch	SMR-8-14	F	60.2	68.9	6.76
Spring Mountain Ranch	SMR-8-14	F	57.0	66.4	5.38
Spring Mountain Ranch	SMR-8-14	F	47.5	54.8	3.36
Spring Mountain Ranch	SMR-8-14	F	53.4	62.4	4.95
Spring Mountain Ranch	SMR-8-14	F	45.4	52.2	3.03
Spring Mountain Ranch	SMR-8-14	F	49.1	56.5	3.66
Spring Mountain Ranch	SMR-8-14	F	54.3	66.0	5.47
Spring Mountain Ranch	SMR-8-14	F	52.5	62.0	4.96

Site	**ID**	**Gender**	**SL**	**TL**	**Wet Mass**
Spring Mountain Ranch	SMR-8-14	F	49.2	57.7	3.73
Spring Mountain Ranch	SMR-8-14	F	44.5	53.2	3.05
Spring Mountain Ranch	SMR-8-14	F	53.5	61.1	4.32
Spring Mountain Ranch	SMR-8-14	F	57.2	64.2	5.24
Spring Mountain Ranch	SMR-8-14	F	50.0	58.3	3.67
Spring Mountain Ranch	SMR-8-14	F	45.2	54.6	3.32
Spring Mountain Ranch	SMR-8-14	F	48.6	57.9	3.92

Notes: ID is code for *Site-Month Collected-Year Collected*. Blanks indicate data missing or omitted due to error.