University of Nevada, Reno

Population genetics and population dynamics of Moapa dace

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

by

Danielle M. Hereford

Dr. Mary M. Peacock/Thesis Advisor

May, 2014
We recommend that the thesis prepared under our supervision by

DANIELLE M. HEREFORD

etitled

Population genetics and population dynamics of Moapa dace

be accepted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Mary M. Peacock, Ph.D., Advisor

Guy A. Hoelzer, Ph.D., Committee Member

James S. Sedinger, Ph.D., Graduate School Representative

David W. Zeh, Ph.D., Dean, Graduate School
ABSTRACT

The Mojave Desert is an arid environment where precipitation ranges from 3.4 to 31.0 cm of rain per year. Species adapted to live in the Mojave Desert tend to be drought resistant and typically utilize little water. Geothermal springs are scare but provide consistent dependable water resources from large carbonaceous aquifers. Springs in the Mojave Desert were manipulated by human populations for agriculture, recreation, or municipalities. As a result, aquatic ecosystems and aquatic species were disrupted on many levels. For example, habitat fragmentation limited movement and dispersal of organisms; population isolation constrained meta-population dynamics and gene flow, and non-native species disrupted food webs, trophic interactions, and displaced native species. As water demands continue to increase in Southern Nevada, aquatic ecosystems are at greater risk and need to be carefully managed. Endangered species risk losing genetic variation and evolutionary potential when habitat is fragmented, limited, or both. Restricted habitat can also limit survival of individuals, recruitment within a population, and the size of a population. This study quantifies genetic variation, population structure, and population dynamics of Moapa dace *Moapa coriacea* in its restricted and fragmented habitat.

Moapa dace *Moapa coriacea* is a thermophilic cyprinid endemic to the Muddy River and its tributaries in Clark County, NV. Historically, Moapa dace occurred in the upper 16 kilometers of the Warm Springs area- the Muddy River and its tributaries. Moapa dace are drift feeders that have unique physiology and biology adapted to live in warm water with low levels of dissolved oxygen. Populations of Moapa dace have
experienced substantial population declines since they were first described by Hubbs and Miller in 1948. Humans manipulated dace habitat by diverting spring outflows for regional municipalities, agriculture, or recreation. After substantial population declines, Moapa dace were listed as endangered in 1967 and United States Fish and Wildlife Service began purchasing property at spring sources to create the Moapa Valley National Wildlife Refuge to protect Moapa dace habitat. Moapa dace populations increased, but later declined when a downstream diversion dam was removed and introduced the non-native piscivore blue tilapia. A gabion barrier was installed in 1997 at the confluence of the Apcar tributary and the Muddy River to protect 2.8 kilometers of stream habitat from further tilapia invasion. Moapa dace have been restricted to the Apcar, Pederson, and Plummer tributaries since 1997. The Moapa dace population was around 1000 individuals from 1999 to 2007, then substantially declined to less than 500 in 2008.

From October 2009 to September 2012 I conducted a bimonthly mark recapture study and estimated Moapa dace abundance, survival, recruitment, and rate of population growth. DNA was extracted from fin clips and ten polymorphic microsatellite loci were used as tags to identify individuals. I also used genetic samples to quantify genetic diversity and population structure among stream tributaries over the three year period.

Moapa dace survival varied by population and over time. Survival was highest in Upper Apcar and lowest in Lower Plummer. Bimonthly Moapa dace survival was highest from February to September and lowest from October to January. Throughout the three year study, annual survival increased by more than 20%. Recruitment was highest in April and June but occurred year round. The rate of population growth declined in Upper
Pederson, Upper Plummer, and Lower Plummer in 2011, but increased in Upper Apcar and Lower Apcar. In 2012, the rate of population growth increased in all populations except Upper Plummer where population growth was not detected in 2012. Over the three year period, increases in Moapa dace population growth was more influenced by increases in survival than increases in recruitment.

Moapa dace were genetically diverse. Allelic richness and heterozygosity were high ($R_s = 11.05 \pm 6.03$, $H_E = 0.70 \pm 0.23$) while the effective population sizes was very low in Apcar ($N_e = 22 - 34$) and Pederson ($N_e = 21 - 71$) and relatively low in Plummer ($N_e = 82 - 104$). A population bottleneck was detected using the heterozygosity excess method, supporting snorkel abundance estimates that depicted recent population declines between 2007 and 2008. Bayesian genotype clustering analysis identified three distinct populations that are separated by current or historic barriers. Significantly different $F_{ST}$ values support the Bayesian genotype clustering analysis depicting limited gene flow in this fragmented stream habitat. The distinct genetic signature depicted in Pederson has been retained from a translocation that occurred in 1984. High levels of genetic variation suggest that evolutionary potential remains high, but low effective population sizes per tributary and geographically distinct populations also suggest that erosion of existing variation due to drift remains a possibility. Management efforts should be aimed at removing barriers and expanding available habitat for Moapa dace to allow for gene flow and maintenance of genetic variation.

This study is the first to quantify the genetic diversity of Moapa dace. It adds to the growing literature of conservation genetics and specifically identifies how habitat
fragmentation has resulted in sub-population differentiation. It is also the first robust population dynamics analysis of Moapa dace survival, recruitment, and rate of population growth. In this study, I establish essential baseline information that will be crucial for assessing the effectiveness of current and future conservation efforts.
ACKNOWLEDGEMENTS

This project was funded by the Southern Nevada Public Land Management Act under project number FW18. Many organizations; U.S. Fish and Wildlife Service, Southern Nevada Water Authority, U.S. Geological Society, Nevada Department of Wildlife, Bureau of Land Management, Moapa Valley Water District, Coyote Springs Investment, Moapa Band of Paiutes, The Nature Conservancy, and Clark County have all extensively contributed to making this project possible and I am exceptionally grateful for this opportunity and experience. Thank you Lee Simons and Amy LaVoie at the U.S. Fish and Wildlife Service for solidifying funding for this project, providing logistical support, sharing data and ideas, and always being amazing advocates for Moapa dace. Thanks also to Alison Manwearing of USFWS who often adapted her daily duties around our field sampling and provided comic relief and friendship in the heat of July. Thank you to the Southern Nevada Water Authority, specifically Dave Syzdek, for providing logistical support and reliable property access.

This project would not have been possible without the hard work ethic from many field partners at U.S. Geological Society. Mark Hereford, Mark Fabes, Antonio Salgado, Paul Bennetts and Gary Scoppettone all contributed muscle, blood, sweat, and exceptional work ethic to this project and I am forever grateful to them. I also thank Gary Scoppettone and Pete Rissler at U. S. Geological Society for designing the field methods for this project.

In the lab, Veronica Kirchoff taught me methods for DNA extraction, polymerase chain reactions, and fragment analysis. Veronica’s patience and problem solving skills were crucial for the timely completion of this project. I am also grateful for her friendship
and kindness. Other students in the Peacock Lab added critical feedback, friendship, and academic support and I particularly thank Kelly Klinger, Jason Smith, and Andy Hickey. Fellow students in the Biology Department, Natural Resources and Environmental Science Department, and Evolution, Ecology, and Conservation Biology Department also provided helpful feedback, input, and friendship. Disproportionately, Dan Gibson provided priceless feedback and advice for population modeling and I am very grateful to him.

My committee has contributed on many levels to my academic career. Thanks to Guy Hoelzer for teaching me population genetic theory and for enthusiastically discussing project results. A special thanks to Jim Sedinger for welcoming a fish biologist into the riveting realm of population dynamics, for providing a foundation and spring board into this field, and for actively encouraging me to be thoughtful and methodical in my analyses. I am very grateful for Jim’s patience and willingness to discuss the best way to incorporate parameters into my models. My major advisor Mary Peacock has been instrumental at every step, from discussing project ideas, population genetic analyses, to results and conclusions. A huge thanks to Mary for her patience, kindness, enthusiasm, and exceptional editing support.

Russell Perry and Ted Jones of U.S. Geological Society also provided database quality control, beneficial modeling feedback, advice, and professional insight, and I am extremely grateful for all that they have taught me and inspired me to do. I especially thank Gary Scoppettone at U.S. Geological Society for giving me the opportunity to work extensively on this project, and for teaching me how to be an active and effective advocate for native fishes. Gary, you have always been an exceptional role model, field
partner, boss, and friend. I am thankful for all the time you have invested in me to help me develop as a biologist.

Finally, I thank my family – my mom and dad, Cindy and Don, my sisters - Samantha and Maya and grandmother Janine, for their patience, emotional and financial support, for their guidance, humor, and thoughtful advise. I also thank my running partner S.Solstice for his steadfast support and enthusiasm. Most of all, I thank my incredible husband Mark Hereford. I am thankful for his support in all aspects of life and this project and I am so grateful for the team that we have become and will continue to be.
# TABLE OF CONTENTS

Title Page  
Signature Page  
Abstract i  
Acknowledgements v  
Table of Content viii  
List of Tables ix  
List of Figures x  
Chapter 1. Population genetics of Moapa dace  
Introduction 1  
Methods 8  
Results 14  
Discussion 19  
Literature Cited 38  
Chapter 2. Population dynamics of Moapa dace  
Introduction 67  
Methods 69  
Results 80  
Discussion 85  
Literature Cited 97
LIST OF TABLES

Chapter 1

Table 1. The primers and their sources used on Moapa dace samples for genetic analysis.

Table 2. Primer concentrations used in polymerase chain reactions for Moapa dace.

Table 3. Number of samples (n), number of alleles (Na), allelic richness (RS), inbreeding coefficient (FIIS), gene diversity (HE), and observed heterozygosity (HO) for each locus and population of Moapa dace.

Table 4. Number of private alleles per population in Apacar, Pederson, and Plummer tributaries.

Table 5. Percent of molecular variance of Moapa dace.

Table 6. Pairwise FST values of Moapa dace.

Chapter 2

Table 1. The primers and their sources used on Moapa dace samples for genetic analysis.

Table 2. Summary of general, temporal, spatial, and individual covariate models.

Table 3. Performance of Pradel survival and recruitment models of Moapa dace detection (p), survival (φ), and recruitment (f).

Table 4. Models of lambda using Pradel recruitment and lambda models for Moapa dace.
LIST OF FIGURES

Chapter 1

Figure 1. Regional map depicting the current hydrology of southeastern Nevada. The study site is part of the Warm Springs area.

Figure 2. Study site for Moapa Dace is comprised of three streams; Apcar, Pederson, and Plummer.

Figure 3. (A) The natural log of the probability of the data and (B) the delta-K method.

Figure 4. Bayesian clustering of Moapa dace.

Figure 5. Population STRUCTURE of Moapa dace captured between October 2009 and September 2012 projected onto the landscape.

Figure 6. Average Bayesian clustering summary of ten iterations of $k = 5$ genotype clusters.

Figure 7. Delta-k for k genotype clusters for Moapa dace in (A) 2010, (B) 2011, and (C) 2012.

Figure 8. Within tributary delta-k and STRUCTURE output for Apcar (A), Pederson (B) and Plummer (C) in 2012.

Figure 9. (A) Annual average Lincoln-Peterson population estimate ($N_c$), (B) genetic effective population size ($N_e$), and (C) $N_e/N_c$ ratio.

Chapter 2

Figure 1. Current hydrology of the Muddy River in Clark County, Nevada.

Figure 2. Detail map of study site and current range of Moapa dace.

Figure 3. Bimonthly capture probability of Moapa dace.

Figure 4. Bimonthly survival of Moapa dace.

Figure 5. Moapa dace bimonthly survival as it varied with fork length- $FL^2$ (A) and heterozygosity at ten loci (B). Number of individuals of Moapa dace captured within different size classes (C) and their heterozygosities (D) at ten loci.
Figure 7. Annual survival probability of Moapa dace.

Figure 8. Seasonal recruitment of Moapa dace.

Figure 9. Annual rate of population growth of Moapa dace populations.
CHAPTER 1. POPULATION GENETICS OF MOAPA DACE

INTRODUCTION

Habitat fragmentation creates isolation among neighboring populations (McMahon et al. 2012) by limiting dispersal (Bélisle et al. 2001; Lu et al. 2012) as well as decreasing the amount of available habitat for organisms within an ecosystem (Saunders et al. 1991; Andrén 1994; Laurance et al. 2011; Proctor et al. 2012), which can truncate biodiversity within habitat patches (Hansen et al. 2005; Ewers and Didham 2006; Carrete et al. 2009). Habitat fragmentation can take many forms both natural and anthropogenic. The former includes naturally fragmented ecosystems such as mountain tops, islands, or meadows and ecosystems that have been temporarily disturbed by a natural event such as wildfire, wind falls, or formation of oxbow lakes or waterfalls. While anthropogenic fragmentation includes roads, cities, agriculture, water diversions, dams, and culverts (Banks et al. 2013; Gibson et al. 2013; Whiteley et al. 2013). Habitat heterogeneity - mountain tops, meadows, islands, - together with natural disturbances contribute to the mosaic of the landscape. Natural disturbances can lead to seasonal or spatial succession within ecosystems – such landscapes are quickly recolonized by wildlife (Banks et al. 2013). Alternatively, anthropogenic features are usually permanent, well maintained structures that can lead to a decline in biodiversity (Ewers and Didham 2006; Hansen et al. 2005; Carrete et al. 2009), enduring population subdivision (Morita and Yamamoto 2002; Dutta et al. 2013; Remon et al. 2013), reduced population size (Fisher et al. 2013; Hoehn et al. 2013), losses in genetic variation (Ohnishi et al. 2007; McMahon et al. 2012; Fitak et al. 2013), and increased extinction risk (Újvári et al. 2002;
Cushman 2006; Chang et al. 2012; Ewers et al. 2013; Levy et al. 2013). While some species appear to be more resilient to habitat fragmentation (Rytwinski and Fahrig 2013), many others have been negatively impacted by habitat fragmentation especially reductions in habitat that are associated with human activity (Gaona et al. 1998; Kyle and Strobeck 2001; Dowling et al. 2005; Hansen et al. 2005; Laurance et al. 2011; Horreo et al. 2013; Kolleck et al. 2013). Due to habitat requirements and diverse life history strategies, the extent that habitat fragmentation affects each species is variable (Andrén 1994; Fahrig 2002; Cushman 2006; Ewers and Didham 2006; Hansen et al. 2005; Hamer and McDonnell 2008). And while some effects of habitat fragmentation are quickly recognized (barriers to movement, mortality from roads, decreases in abundance) (Hansen et al. 2005); others, such as losses in genetic variation may take several to hundreds of generations to realize (Spencer et al. 2000; England et al. 2003). Population isolation, caused by disturbance and habitat fragmentation, have resulted in losses of genetic diversity and increased genetic differentiation among populations (McMahon et al. 2012; Banks et al. 2013).

Small populations tend to have less genetic variation (Ohnishi et al. 2007; Whitely et al. 2010; Furlan et al. 2012; Peacock and Dochtermann 2012; Fitak et al. 2013) and smaller effective population sizes (Leberg 2005; Pertoldi et al. 2007). The effective population size ($N_e$) is a measure that is directly linked to the amount of genetic variation in a population and $N_e$ determines the rate that genetic variation is lost, consequently, it is considered to be one of the most informative parameters for assessing long term species persistence (Pertoldi et al. 2007; Osborne et al. 2010). Populations with
low genetic variation are more limited in their ability to evolve and are more vulnerable
to stochastic environmental and demographic events (Reed and Frankham 2003; Pertoldi
et al. 2007; McMahon et al. 2012). Many studies of genetic diversity in imperiled species
suggest that short term population census size ($N_c$) decline does not always result in
significant losses in genetic variation (Yamamoto et al. 2007; Osborne et al. 2010; Zhu et
al. 2013); however, local or permanent extinction is a real threat to many species that
have been subjected to long term isolation and reduced population sizes (Saunders et al.
1991; Gibson et al. 2013; Munguia-Vega 2013). Therefore, maintaining and monitoring
genetic diversity is a top conservation priority to ensure populations of concern are
diverse enough to evolve and withstand stochastic events (Pertoldi et al. 2007; McMahon
et al. 2012). By understanding how fragmentation has altered the genetic diversity and
genetic structure of a species, managers can implement strategies that best suit vulnerable
species and conserve genetic variation.

Habitat fragmentation is especially common in fresh water stream and river
systems because of the overarching anthropogenic demand for this essential resource
(Geist 2011). Estimates ranging between 10,000 - 20,000 freshwater species have been
jeopardized or are extinct due to habitat fragmentation (Strayer and Dudgeon 2010).
While fresh inland water makes up < 1% of the earth’s surface area, freshwater and
diadromous fishes make up one-third of all vertebrate biodiversity worldwide and 43% of
all fishes (Burkhead 2012). Many freshwater aquatic ecosystems have been negatively
impacted by fragmentation due to agriculture diversions, impoundment, flow regulation,
pollution, habitat degradation, invasive species introductions, over exploitation, and
urban development (Harding et al. 1998; Strayer and Dudgeon 2010; Geist 2011; Roberts et al. 2013). Much of the biodiversity that has evolved in riverine systems is dependent upon the dynamic, highly transient nature of fluvial systems and the inhabitants rely on the heterogeneous habitat that river systems provide (Ward and Tockner 2001; Dudgeon 2010). Anthropogenic river manipulation has fragmented the habitat and eliminated much of the natural habitat heterogeneity (Ward 1998; Geist 2011). For many species, the remaining patches may not be large enough contain the diverse habitat features necessary for a species to fulfill its life history potential (Whiteley et al. 2013). Likewise, shifts in the physical environment impose drastic changes to the biodiversity, food web dynamics, and ecosystem function of an aquatic system (Dudgeon 2010). Major disruption in these processes can lead to extinction or even ecosystem collapse (Dudgeon 2010). Extinction rates for freshwater fishes in North America are exceptionally high; 877 times higher than the background extinction rate (Burkhead 2012). In North America, 57 species and subspecies went extinct between 1900 and 2010 because of anthropogenic perturbations (Burkhead 2012). Understanding the effect of fragmentation on a species is especially important for endangered fishes because the species existence may be dependent upon restoring connectivity and ecosystem function (Dudgeon 2010). Stream habitat fragmentation has led to the extinction of fish species such as the Tecopa Pupfish and the Alvord Cutthroat Trout (Burkhead 2012). Hundreds of other freshwater fishes have been listed as either threatened or endangered as a result of human induced alterations to the environment (Dudgeon 2010). The consequences of anthropogenic manipulation are not only grave for freshwater fishes and their ecosystems, but also for humans (Tedesco et al. 2013). Freshwater ecosystems clean water supplies, support all walks of life, and are
crucial for the economy of every human development (Geist 2011; Tedesco et al. 2013). The necessity of healthy freshwater ecosystems is not easy to overstate.

The Muddy River in southern Nevada is one of four tributaries that flows into manmade Lake Mead; a reservoir that began impounding water from the Colorado River, the Muddy River, the Virgin River, and the Las Vegas wash in 1935 (Holdren and Turner 2010). Lake Mead is the most important reservoir in the United States because it provides water for about 25 million people, creates almost 3 million horsepower in hydroelectric power, and provides ample recreation for the surrounding human population (Holdren and Turner 2010). Between 1999 and 2009, the elevation of Lake Mead dropped 35 meters, resulting in a 43% decrease in surface area and a 50.3% decrease in volume (Holdren and Turner 2010). As water demands in the arid southwest continue to grow, so does interest in the minor tributaries (the Muddy River, the Virgin River, and the Las Vegas Wash) that feed the reservoir (Holdren and Turner 2010). The Muddy River is formed by more than 20 warm (30-32°C) geothermal springs that emerge from a large carbonaceous aquifer in Southeastern Nevada (Fig. 1) (Scoppettone et al. 1998).

Historically, the upper reaches were extensively modified for recreation and agriculture purposes, and while portions have been recently restored, much of the habitat remains substantially altered from natural conditions (Hatten et al. 2013). Water diversions, dams, ground water pumping, habitat degradation, and non-native species (plant and animal) are prominent features that have altered the Muddy River ecosystem (Scoppettone et al. 1992, Hatten et al. 2013).
Moapa dace *Moapa coriacea* is a small (<120 mm) endangered thermophillic cyprinid endemic to the Muddy River and its tributaries (the Warm Springs area) (Scoppettone and Goodchild 2009). Listed under the United States Endangered Species Act in 1967, it is the only species in its genus (Scoppettone et al. 1992). Moapa dace abundance ($N_c$) was first estimated in 1984 to be 2800 individuals (Scoppettone et al. 1992). Abundance estimates peaked in 1994 when snorkel surveys enumerated 3841 adult Moapa dace (Scoppettone et al. 2005)(Fig. 3). The population experienced a substantial decline in 1995 when a diversion dam was removed, allowing the voracious non-native piscivore Blue tilapia (*Oreochromis aurea*) access to the Warm Springs Area (Scopettone et al. 2005). Since 1995, Moapa dace have been restricted to ten percent of its historic range, about 2.9 kilometers of steam habitat (See Figure 2). Moapa dace are currently only found in three tributaries to the Muddy River; Apcar, Pederson, and Plummer (Fig. 2). These tributaries emerge on the Moapa Valley National Wildlife Refuge (hereafter “Refuge”) from a series of springs. The water cools as it travels downstream and these tributaries converge on the Warms Springs Natural Area (hereafter “Natural Area”), which is owned by Southern Nevada Water Authority (SNWA) (Fig. 2). The Refuge was acquired in parcels between 1979 and 2001 (Scoppettone et al. 1992; Hatten et al. 2013) and was the first wildlife refuge established for the sole protection of a fish species. The Natural Area was acquired by SNWA in 2007 (Galati 2011). Prior to acquisition of the land, these streams had been extensively manipulated for municipalities, agriculture, and recreation. Several chlorinated pools collected the spring water and non-native fishes mosquitofish (*Gambusia affinis*) and short fin molly (*Poecilia mexicana*) have been present in the system since before 1948 and 1963,
respectively (Hubbs and Miller 1948, Hubbs and Deacon 1964). Extensive restoration has converted this former recreational resort to some semblance of the natural ecosystem (Hatten et al. 2013). Snorkel surveys organized by U.S. Fish and Wildlife Service indicate the lowest census (SN<sub>c</sub>) counts of Moapa dace occurred between February 2008 and February 2012 (SN<sub>c</sub> = 459 - 713, \(\bar{S}N_c = 563 \pm 102, n = 9\)) (Fig. 3) (Simons unpublished data). While concrete pools and diversions have been removed from the Refuge and part of the Natural Area, the remaining Moapa dace habitat is still largely fragmented (Fig. 2). All streams are channelized into culverts under Warm Springs Road and have varying degrees of isolation due to other culverts and other poorly understood barriers, such as reduced flows and non-native vegetation barriers (personal observation) (Fig. 2).

Moapa dace has experienced large reductions in habitat (~90%) and population size (~80%) (Scoppettone et al. 1992, Simons unpublished data). Such a population reduction suggests concomitant losses of genetic variation through a genetic bottleneck. In addition, barriers present within the current range of Moapa dace may be a formidable environment for gene flow, resulting in genetic differentiation among existing tributary populations. Genetic assessments of endangered species such as the Moapa dace can be particularly helpful for managers interested in preserving genetic diversity. The aims of this study were to use data collected from nuclear, non-coding microsatellite markers to: i) quantify allelic diversity and heterozygosity, ii) quantify population differentiation using F statistics, iii) identify population structure using Bayesian genotype clustering.
analysis, iv) test for population bottlenecks, v) estimate the genetic effective population size ($N_e$), and vi) estimate changes in these parameters over a three year period.

METHODS

Study site

The Warm Springs Area is located in the Mojave Desert in Clark County, Nevada and is comprised of the United States Fish and Wildlife Service (USFWS) Moapa Valley National Wildlife Refuge property and the Warm Springs Natural Area, property owned and managed by Southern Nevada Water Authority. Three streams emerge from geothermal springs on the refuge and converge on the Natural Area approximately 600-900 meters downstream. These three springs, Apcar, Pederson, and Plummer are part of a large carbonaceous aquifer where several mineral rich seeps emerge from the ground and form the head waters of the Muddy River. Average discharge from the Warm Springs Complex is 1.05 ± 0.11 cubic meters per second (Average ± SD of USGS water gauge 09416000 October 2009- September 2012) where 30-32°C water emerges from these springs and cools as it travels downstream. Because the region has been heavily impacted by anthropogenic disturbances – agriculture, pool resorts, and local municipalities – habitat restoration has been an integral part of creating the wildlife refuge and natural area and has been ongoing since the mid 1980’s (Hatten et al. 2013). Historically, Moapa dace were found throughout the Muddy River until non-native blue tilapia were introduced and decimated the dace population in 1995 (Scoppettone and Goodchild 2009). During this study, Moapa dace were only found in Apcar, Pederson, and Plummer tributaries, in about 2.8 kilometers of habitat above a down-stream gabion barrier.
Several physical barriers are present throughout the stream system (Fig. 2). Moapa dace share their limited habitat with Moapa White River springfish (Crenichthys baileyi), a native poolfish and a variety of non-native species including the American bullfrog (Rana catesbeiana), mosquitofish (Gambusia affinis) and short fin molly all of which have substantially changed the historic dynamics of the stream system. Mosquitofish and short fin molly have been present in the system since before 1948 and 1963, respectively (Hubbs and Miller 1948; Hubbs and Deacon 1964). Native vegetation (mesquite Prosopis sp., ash Franzinua sp., arrow weed Pluchea sericea, Yerba Mansa Anemopsis californica, Willow Salix exigua and Salix gooddingii and Tarragon Artemisia dracunculus) and non-native vegetation (Palm trees Washingtonia filifera and Phoenix dactylifera, fresh water eel grass Vallisneria, Cattails Typha domingensis and Typha latifolia) are present in varying degrees throughout the study area. Distribution of non-natives, physical barriers and historical manipulations are all thought to contribute to observed distribution and population genetics of Moapa dace.

Field sampling

The entire extant range of Moapa dace was surveyed bimonthly over a period of three years between October 2009 and September 2012. Gee minnow traps were set every five or 25 meters in a random stratified sampling regime at 167 established station locations (Fig. 2). The portions of Plummer and Apcar where traps were set every five meters on the refuge were thought to have more dace. Traps were set to increase dace capture probability and to actively remove any non-native fish in these regions. Each trap was set twice per sampling period in a robust design mark recapture framework. The
interval between the primary and secondary sets was always less than four days. The interval between primary sets was about eight weeks. Traps were baited with dry dog food and set for three to twenty eight hours. Duration of set time was dependent upon water temperature and location. Stress induced on Moapa dace was minimized by reducing trap hours in high capture probability areas. While each station was located at a specific distance from the springhead, each trap was selectively placed within two meters of the station to maximize dace capture success. Upon capture, ten random individuals from each species were measured; all other captured fish were identified to species and enumerated. All captured Moapa dace were measured, weighed, and a small sample of their caudal fin was clipped and preserved for individual identification using genetic methods.

Top caudal fins were clipped on the primary occasion and bottom caudal fins were clipped on the secondary occasion. Using two different marks allowed for easy identification of fish that were already captured within that occasion, for example, if a fish was clipped, swam downstream, and into another trap that had yet to be pulled. There were very few instances (n < 10) of recapturing individuals within the same occasion. Two different marks also allowed for utilization of the Peterson-Lincoln population estimate. Initially, caudal fin clips (hereafter fin clips) were stored in one milliliter of 95% ethanol in Eppendorf tubes. Clips stored in ethanol resulted in low quantities of degraded DNA so collection was modified in May 2010 and fin clips were stored in folded wax paper in paper coin envelops and were allowed to dry out completely.

**DNA extractions and genetic analysis**
DNA was extracted, amplified using polymerase chain reaction (PCR) technology and successfully genotyped from 3307 fin clips. For fin clips stored in ethanol, the ethanol was evaporated prior to DNA extraction. DNA was extracted from all fin clips according to manufacturer’s instructions for QIAGEN DNeasy 96 tissue extraction kits for Animal Tissues (QIAGEN 2006) with the exception of the last step where samples were eluted in two steps with 75 µL of 56°C AE buffer. All samples were sealed with Airpore tape, incubated at ~22°C for five minutes and then centrifuged for two minutes. This step was repeated to increase the concentration of DNA obtained for each sample. DNA was quantified using a Labsystems Fluoroskan Ascent fluorometer (Nevada Genomics Center, http://www.ag.unr.edu/genomics/).

A total of ten microsatellite loci were amplified in five multiplex polymerase chain reactions (PCR) (Table 2). PCRs were conducted using TECHME ® TOUCHGENE thermal cyclers. Of the ten loci used in this study, six were developed from Eurasian dace (Leuciscus leuciscus), two from Lahontan Tui Chub (Gila bicolor), one from Roundtail Chub (Gila robusta), and one from Bonytail Chub (Gila elegans) (Dubut et al. 2009; Meredith and May 2002; Keeler-Foster et al. 2004; Dowling et al. 2005) (Table 1).

Five multiplex PCRs (“PCR1”–“PCR5”) were carried out on each sample (Table 2). All loci were initially tested with concentrations of 0.25 µM forward primer, 0.75 µM reverse primer, and 0.25 µM a fluorescent label (VIC, NED, or FAM); these concentrations were adjusted to optimize amplification among primers. Twelve µL reaction volumes contained template DNA (30-70 ng), six µL of 2X QIAGEN Master
Mix, 0.08-0.42 µM of forward primers, 0.25-1.25 µM of reverse primers, and 0.08-0.42 µM of fluorescent label (Table 1 and 2). The PCR conditions for Lsou08, LleC-090, Gbi-G34, Gbi-G39, Gel_223, GilaD17, BL1-2b and Lsou05 (PCR2-PCR5) consisted of a 15 minute hot start at 95°C then 21 cycles of 30 s 95°C with seven 90 s cycles at 65°C, 61°C, 58°C and 30 s at 72°C followed by 20 cycles of 30 s 95°C, 90 s 55°C, 30 s 72°C which added florescent M13 label. PCR conditions for Lce-C1 and LleB-072 (PCR1) were similar except that they consisted of ten cycles at 65°C, 61°C, and 58°C annealing temperatures to increase amplification yield. PCR products were diluted to concentrations that optimized peak intensity for multiplex panels. Fragment analysis was carried out on a Perkin Elmer Applied Biosystems 3730 Genetic Analyzer (Nevada Genomic Center, http://www.ag.unr.edu/genomics/).

All alleles were genotyped using GeneMapper Software (version 3.7) by two independent observers. If discrepancy or uncertainty arose at a particular locus, the locus was removed from analysis. Individuals that failed to amplify at more than five of the ten loci were removed from analysis. GENECAP, an excel macro, was used to identify individuals by calculating allele frequencies, quantifying probability of identity, and matching genotypes (Wilberg and Dreher 2004). GENECAP calculates the probability that two individuals have the same genotype assuming: (1) Hardy Weinberg equilibrium using methods described by Paetkau and Strobeck (1994) - $HW \ P(ID)$, and (2) under the assumption that individuals could siblings; the probability of sibling identity- $SIB \ P(ID)$, as described by Waits et al. (1999). All $SIB \ P(ID)$ values that were >0.05 were omitted from analysis. GENECAP allows for samples with incomplete genotypes to be included
for individual identification, however if alternating loci are missing adequate genetic information, GENECAP identifies these incomplete genotypes as ‘problem’ samples that must be removed from the analysis. Finally, individual covariates such as fork length (mm) and mass (g) were correlated with genotype capture history to identify mismatches.

Data analysis

For all annual analyses, the 18 sampling occasions were equally divided into three temporal sections; 2010 = October 2009-September 2010, 2011 = November 2010-September 2011, 2012 = November 2011-September 2012. Microchecker (version 2.2.3; Van Oosterhout et al. 2004) was used to detect null alleles and to test for allelic dropout. Any locus with null alleles present in all populations was removed from the analysis. Microsatellite toolkit, an excel macro, was used to calculate allele frequencies and both observed and expected heterozygosities for all loci (Piry et al. 1999). FSTAT was used to quantify F statistics to test for genetic differentiation among subpopulations (Goudet 1995; Goudet 2001). GenAlEx (version 6.5; Peakall and Smouse 2012) was used for analysis of molecular variance (AMOVA). A Bayesian genotype clustering approach (STRUCTURE v 2.3.3; Pritchard et al. 2000) was used to infer population structure from microsatellite data for each year of the study. For each potential genotype cluster (k), a 500,000 iteration burn-in period and ten 500,000 Markov Chain Monte Carlo replicates were specified (k = 1-12). The optimal number of genotype clusters was assessed using STRUCTURE Harvester (Earl and vonHoldt 2012) and the Evanno et al. (2005) Δk method. STRUCTURE clustering results informed the decision to group all analyses into three subpopulation groups; Apcar, Pederson, and Plummer (Fig.5) A Stepwise Mutation
Model (SMM) and Two Phase Mutation model (TPM) in the program BOTTLENECK were used to test for genetic bottlenecks (Piry et al. 1999). NeEstimator (version 2; Do et al. 2014) was used to estimate the effective population size (\(N_e\)) using the linkage disequilibrium module based on a single point sample for each tributary (Do et al. 2014). The bias-adjusted Lincoln-Peterson population estimator was used to estimate Lincoln-Peterson population size (\(LPN_c\)) for each system within each bimonthly sampling occasion using detection of fin clip as recapture (Williams et al. 2002). Annual average \(LPN_c\) was used to estimate population census size, \(N_c\). Annual \(N_e\) and \(N_c\) estimates were used to calculate \(N_e/N_c\) ratios. \(LPN_c\) from January and March, and July and September were averaged and compared to snorkel abundance estimates (\(SN_c\)) performed annually in February and August by the Muddy River Biological Advisory Committee. To directly compare our \(LPN_c\) estimates to regularly conducted snorkel abundance estimates from USFWS, we used slightly different population boundaries; the lowest section of Pederson below the second barrier and before the confluence is included with Pederson. For all other analyses, including other \(LPN_c\) estimates (e.g. in \(N_e/N_c\) ratios) the population designations described in Fig.5 were used. The August 2009 snorkel survey was not compared to any \(LPN_c\) because this was prior to the inception of this study. In addition, larval Moapa dace were removed from snorkel abundance estimates for these comparisons (Simons unpublished data).

RESULTS

Genetic Variation

Null alleles were present at two of the ten microsatellite loci (Gel_223 and Gbi-G39) in all populations of Moapa dace sampled and were therefore removed from all
further analyses. Of the eight remaining loci, the average number of alleles per locus per population was 13.5 ± 6.2 (SD) with a range of 4 to 22 alleles per locus per population ($N_A$) (Table 3). There was no significant difference in the number of alleles per locus among populations for all years combined ($\chi^2 = 0.153$, df = 2, $p > 0.46$). Pederson had the fewest number of total alleles (80), followed by Apcar (89), while Plummer had the most alleles (104). However, these observed differences in total number of alleles among populations, all years combined were not significantly different ($\chi^2 = 3.23$, df = 2, $p > 0.20$). Although 104 alleles were detected in the first year of the study (n = 409 individuals captured), only 95 (n = 686), and 88 (n = 865) were detected in 2011 and 2012 respectively despite an increase in sample size. There were two alleles not detected in 2010 that were detected in 2011 and one additional allele was detected in 2012 that was not in either of the previous sampling periods. The observed reduction in number of alleles between 2010 and 2012 was not statistically significant ($\chi^2 = 1.33$, df = 1, $p > 0.24$). Of the 16 alleles putatively lost over the three year study, three were from Apcar, one was from Pederson, and 13 were lost from the Plummer stream (Table 4). All alleles putatively lost were rare (frequency ≤ 0.008) and therefore may still be in the population but not sampled.

Over all three years of the study, allelic richness ranged from 2.5 to 19.84 per locus per population, with an average of 11.05 ± 6.03 (SD) (Table 3). Allelic richness at any one locus did not differ among tributaries when all years were combined ($\chi^2 = 0.81$, df = 2, $p > 0.66$) and did not differ among years when all tributaries were combined ($\chi^2 = 0.12$, df = 2, $p > 0.93$). Expected heterozygosity (HE) ranged from 0.17- 0.93
(average ± SD = 0.70 ± 0.23; Table 2). Expected heterozygosity did not differ among tributaries ($\chi^2 = 0.02$, df = 2, p > 0.99) and did not differ among years ($\chi^2 = 0.01$, df = 2, p > 0.99). Observed Heterozygosity (Ho) ranged from 0.16 - 0.96 (average ± SD = 0.70 ± 0.24). There was no significant difference in observed heterozygosity among tributaries ($\chi^2 = 0.03$, df = 2, p > 0.98) or among years ($\chi^2 = 0.01$, df = 2, p > 0.99). There was no departure from Hardy Weinberg equilibrium among populations and loci, $F_{IS}$ values ranged from -0.19 to 0.12 (Table 3). Genetic bottlenecks were detected in Apcar ($p = 0.004$), Pederson ($p = 0.03$), and Plummer ($p = 0.03$) under the TPM model and Wilcoxon sign rank test.

**Population structure analysis**

AMOVA conducted for all individuals captured between October 2009 and September 2012 revealed ~ 0.3% of the molecular variance observed was due to differences among all six populations, 1% of the molecular variance was partitioned among streams, 12% of molecular variance was explained by differences among individuals, and 86% of the molecular variance was explained within individuals. AMOVAs of individuals captured in 2010 or 2012 showed similar partitioning of molecular variance to the overall AMOVA. In contrast, in 2011 0.5% of variation was due to differences among all six populations, 1.5% of the molecular variance was due to differences among stream systems, 6.2% of the molecular variance was explained by among individual variation, and 92% of the molecular variation was within individual variation. (Table 5).
The Bayesian genotype clustering analysis for all Moapa dace (n = 1557) captured between October 2009 and September 2012 identified \( k = 2 \) and 5 genotype clusters as the best fit of the data (Fig. 3). In the \( k = 2 \) analysis the fish in Apcar and Pederson tributaries were more similar to each other than to fish in Plummer (Fig. 4a). Whereas in the \( k = 5 \) analysis all three tributaries were distinct (Fig. 4b).

Genetic differentiation among populations coincides with current and historic manmade barriers (Fig. 5). In the \( k = 5 \) analysis, the red cluster predominated in Upper and Lower Apcar at 44.2 and 35.7% respectively (Fig. 6). The blue (44.6 and 48.4%) and pink (34.5 and 32.2%) genotype clusters were dominant in Upper and Lower Pederson (Fig. 6). No single genotype cluster had greater than 30% representation in Upper or Lower Plummer but the green (27.0% and 28.2%) and yellow (28.8% and 29.8%) clusters were predominant in these reaches (Fig.6). When analyzed by year, Bayesian genotype cluster analysis displayed similar clustering patterns of individuals, though the number of clusters that best fit of the data varied by year; 2010 \( (k = 4) \) 2011 \( (k = 3) \) and 2012 \( (k = 2) \) (Fig. 7). Bayesian cluster analyses for each reach and year revealed no detectable substructure within tributaries (Fig. 8).

Pairwise F\(_{ST}\) values among tributaries with all years combined ranged from 0.0096 - 0.0252 and were all significantly different than zero (p = 0.0167) (Table 6). These results supported the STRUCTURE cluster analysis in suggesting that there are three distinct subpopulations of Moapa dace in the Warm Springs area. Apcar and Plummer were more similar to each other (0.0096) than either Apcar (0.0204) or Plummer (0.0252) was to Pederson (Table 6). Between 2010 and 2012, F\(_{ST}\) values among
tributaries increased in all pairwise comparisons (Apcar-Pederson; 0.0162, 0.0188, 0.0229 Apcar-Plummer; 0.0079, 0.0113, 0.0113 Pederson-Plummer; 0.0185, 0.0253, 0.0274.) supporting significant differentiation among tributaries. All annual $F_{ST}$ values among systems were significantly different from zero.

**Census size, effective population size, and $N_e/N_c$ ratio**

The population estimate ($L\!P_{Nc}$) using bimonthly Lincoln Peterson mark-recapture ranged from 13-254 in Apcar, 26-242 in Pederson, and 120-485 in Plummer over all 18 sampling periods. The $L\!P_{Nc}$ in Apcar increased from 36 in 2010 to 92 in 2011 and 189 in 2012 (Fig. 9a). Average $L\!P_{Nc}$ was dynamic in Pederson (2010 = 47, 2011= 138, 2012 = 100) and relatively stable in Plummer (2010 = 264, 2011 = 311, 2012 = 266) (Fig. 9a).

Snorkel abundance estimates ($S\!N_{c}$) from February and August were higher than the average of $L\!P_{Nc}$ in January and March or July and September respectively in Pederson and Plummer on every capture occasion, except for August 2011 when $S\!N_{c}$ estimates were lower in Pederson. On average, $S\!N_{c}$ estimates were greater than $L\!P_{Nc}$ estimates by 75 in Pederson and 104 individuals in Plummer and by 20 individuals in Apcar.

The genetic effective population size ($N_e$) estimated for each year between 2010 and 2012 for Apcar and Pederson tributaries was found to be very low, although $N_e$ increased in Apcar and was stable in Pederson over the course of the study (Apcar, 2010 = 22, 17-29 95% CI; 2011 = 29, 24-35 95% CI; 2012 = 34, 29-40 95% CI: Pederson, 2010 = 72, 41-189 95% CI; 2011 = 21, 17-26 95% CI; 2012 = 21, 17-2595% CI) (Fig.
9b). The $N_e$ in Plummer was much higher, 104 (81-173 95% CI), 82 (69-98 95% CI), and 94 (78-114 95% CI) in 2010, 2011 and 2012 respectively (Fig. 9b).

Over the three year study, the $N_e/N_c$ ratio declined substantially in Apcar (2010 = 0.62, 2011 = 0.32, 2012 = 0.18) and increased in Pederson (2010 = 1.52, 2011 = 0.15, 2012 = 0.21) (Fig. 9c). In comparison, $N_e/N_c$ was more stable in Plummer (2010 = 0.39, 2011 = 0.26, 2012 = 0.35) (Fig. 9c).

**DISCUSSION**

**Detection of bottlenecks**

One objective of this study was to identify whether Moapa dace have experienced population bottlenecks given the extensive habitat fragmentation and modification. Small populations that have recently endured population bottlenecks tend to have higher amounts of allelic diversity and higher levels of heterozygosity than expected for a population its size (Peery et al. 2012). The genetic result showing evidence of bottlenecks was supported by the trend seen in biannual snorkel abundance surveys (Simons, unpublished data). The genetic signature of excess heterozygosity and high allelic diversity suggests that the bottleneck being detected can be attributed to the most recent observed population decline between 2007 and 2008. Historical accounts such as the 1994 fire and the invasion of blue tilapia (Scoppettone et al. 1998) suggest that Moapa dace have experienced bottlenecks prior to the 2007-2008 but it is likely that the genetic signature of these bottlenecks has been masked by the most recent population decline. The amount of allelic diversity a population can maintain after a bottleneck is dependent
upon the severity and the duration of the bottleneck (Spencer et al. 2000). If a population is able to rebound quickly post bottleneck, the loss of allelic diversity could be minimal or moderate (Antao et al. 2011). Snorkel abundance surveys suggest that abundance of Moapa dace has increased since 2008, albeit slowly (Simons, unpublished data). Short, very severe bottlenecks have been shown to reduce allelic diversity to a greater extent than long diffuse bottlenecks while heterozygosity was reduced equally (Spencer et al. 2000; England et al. 2003). Populations that are unable to rebound post bottleneck suffer from the effects of genetic drift such as excess homozygosity, reduced genetic diversity, and a lower effective population size, thereby reducing their evolutionary potential and increasing risk of extinction (Cornuet and Luikart 1996; Frankham 1996; Antao et al. 2011).

**Census size**

Closed population estimates using capture-mark-recapture and the Lincoln Peterson population estimator ($\text{LPN}_c$) were variable for each tributary, ranging from 13-254 in Apcar, 26-242 in Pederson, and from 120-485 in Plummer. The confidence intervals for some $\text{LPN}_c$ estimates were relatively large because overall capture probability was relatively low and the biology of the fish limits detection by this sampling method (Scoppettone1993; Luikart et al. 2010). $\text{LPN}_c$ estimates did not display any seasonal trends but did oscillate over the three year study. To analyze change in $\text{LPN}_c$ over the three year study, average $\text{LPN}_c$ estimates were used to estimate annual average population estimate ($N_c$), which provided a conservative estimate that diminished the effects of any biased high or low estimates due to capture heterogeneity or change in
behavior due to trap response (Williams et al. 2002; Luikart et al. 2010). In addition, this ensured that the same sampling regime and dataset was used for \( N_c \) and \( N_c \) estimates. \( N_c \) in Apcar increased from 36 in 2010 to 92 in 2011 and up to 189 in 2012. \( N_c \) was highest in 2011 in Pederson \((2010 = 47, 2011 = 138, 2012 = 100)\) and Plummer \((2010 = 264, 2011 = 311, 2012 = 266)\) (Fig. 9). SNWA finished restoration in 2011 on Apcar near the Pederson confluence (Syzdek, personal communication). Restoration focused on creating better habitat for Moapa dace by increasing the flows, narrowing the channel, and establishing habitat necessary for better foraging opportunities (Syzdek, personal communication). The decrease in \( N_c \) in Pederson and Plummer and subsequent increase in \( N_c \) in Apcar can at least in part, be attributed to the movement of individuals from Pederson and Plummer into the newly restored habitat in Apcar. It is probable that the restoration increased the carrying capacity of Apcar, which increased the population size in Apcar.

On average, LPN\(_c\) estimates were lower than snorkel abundance estimates (SN\(_c\)) in Pederson and Plummer, by 75 and 104 individuals and more than half of snorkel abundance estimates were outside of the LPN\(_c\) 95% CI. In Apcar, these estimates were more similar (mean difference = 20.4 individuals) and all but one SN\(_c\)s was larger than the 95% CI of both LPN\(_c\)s. Most of the observed variation is likely due to different sampling occasions (e.g.: SN\(_c\) = February, LPN\(_c\) = mean LPN\(_c\) for January and March) or sampling technique. SN\(_c\) may be higher than LPN\(_c\) because some smaller fish may be more likely to evade minnow trap capture than larger fish, but may be equally detected by experienced snorkelers. LPN\(_c\) in Pederson is expected to have the largest confidence
intervals because fewer traps were set in this section (Fig. 2), leading to lower capture probability and less precision. Still, LPN_e and SN_e estimates follow the same general trend, no estimates are egregiously different, and LPN_e is a more conservative estimate. For all further analyses, LPN_e estimates based on populations from Fig. 5 were used because these estimates were derived from the same data set as N_e estimates.

Snorkel surveys have been used extensively in the Muddy River system but their utility has not been directly compared to other estimators like mark recapture within the same time period (Scoppettone et al. 1992; 1998). While MRBAC snorkel estimates were higher than mark recapture estimates, others have shown snorkel estimates to be biased low especially for fish in smaller size classes citing time of day, water temperature, and visibility as other factors that affect detection rates (Thurow et al. 2006). Water temperature in the Muddy River and its tributaries is warm (28 - 32°C), and the biology of dace is much different than that of trout, the subject of the Thurow et al. (2006) study, so their response behavior to snorkeler presence is different. Moapa dace have conspicuous behavior, feeding in the water column in large groups of > 10 individuals so it is likely that MRBAC snorkel estimates are a reasonably accurate measure of N_e (Scoppettone et al. 1992). These snorkel estimates are highly valuable because SN_e estimates can be compared to historic snorkel surveys.

Large population fluctuations are inherent in many fishes because their reproductive biology produces many gametes that have high mortality in early life (Helfman et al. 2009). Moapa dace snorkel estimates peaked in 1994 when more than 3800 individuals were detected in the Muddy River and its tributaries (Scoppettone et al.
Snorkel censuses note that up to 49% of abundance was in the Muddy River; and other springs such as Muddy Spring and the South Fork, harbored substantial populations of Moapa dace, ranging from 100-1450 individuals each in 1984, 1986, and 1987 (Scoppettone et al. 1992). Adult Moapa dace were found to be significantly larger and therefore more fecund in the Muddy River than in the tributaries (Scoppettone et al. 1992). It is likely that dace $N_c$ would increase dramatically if the Muddy River and other tributaries become habitable. At this time, rotenone treatments for tilapia eradication (most recent 2012), appear to have been effective (Simons, personal communication). Until the Muddy River and its tributaries are deemed Tilapia-free, the continued enhancement of habitat on MVNWR and WSNA is likely to be the most effective way of increasing $N_c$.

Recovery plans for endangered species typically include a target population size that is derived from census estimates. The recovery plan for Moapa dace states that Moapa dace will be considered for delisting when Moapa dace inhabit at least 75% of their historical habitat in the Muddy River and its tributaries, and at least 6,000 adult Moapa dace are present for five consecutive years (USFWS 1995). Many studies have shown that population size only weakly describes evolutionary potential, and therefore extinction risk (Palstra and Ruzzante 2008). When managing an endangered species, it is always best to use the lower, more conservative population estimate because the risks associated with managing a population with an artificially high estimate could be detrimental to the species. This is especially true for species like Moapa dace that can experience substantial population fluctuations within a year. If an endangered population
is perceived to be more stable than it actually is, time sensitive management could be postponed, which could unnecessarily increase extinction risk (Schwartz et al. 1998).

**Effective population size**

Throughout the last century, the Warm Springs Complex has sustained substantial changes in the flow regime which has had differential effects on the abundance and genetic diversity of Moapa dace through time. Even though Moapa dace were found to be relatively abundant by Hubbs and Miller in 1938 (Hubbs and Miller 1948) and by Scoppettone et al. in 1994 (Scoppettone et al. 1998), the amount of neutral genetic diversity that persists despite repeated ecosystem and population perturbations is remarkable. Plummer had surprisingly high \( N_e \); 104, 82, and 94 in 2010, 2011, and 2012. In contrast, Apcar and Pederson had markedly low \( N_e \) (\( N_e = 22-34.2 \) and \( 20.6-71.8 \), respectively) which should be viewed as a red flag for managers to implement proactive population management. Effective population size is a valuable measure of genetic diversity because it can be used to understand the amount of genetic variation, the rate of loss of genetic variation, the evolutionary potential, the demographic status, and the rate of genetic drift (Kalinowski and Waples 2002; Luikart et al. 2010). \( N_e \) estimates tend to be biased low for species with high reproduction and low survivorship or if \( N_e \) estimates are made using small sample sizes or data with overlapping generations (Palstra and Ruzzante 2008). High reproduction and low survivorship are part of the biology of Moapa dace so \( N_e \) estimates reported here may be low. Small sample size only affected the \( N_e \) estimate in Pederson in 2010 (\( N_e = 71.8 \) 95% CI= 40.8-189.1) and it is expected that the influence of overlapping generations is negligible due to low survivorship of
individuals (Scoppettone et al. 1992). Severe population declines, low $N_e$s, and population bottlenecks are exceptionally prevalent in desert fishes (Dowling et al. 2013). Owen’s Valley Pupfish (*Cyprinodon radiosus*), Mohave tui chub (*Siphateles bicolor mohavensis*), Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*), are all desert fishes that have undergone population bottlenecks and have reduced $N_e$s that are attributed to habitat fragmentation (Finger et al. 2013; Chen et al. 2013; Neville et al. 2006). The general consensus is that populations with an effective population size of less than 50 are very vulnerable to the effects of inbreeding depression (Frankham et al. 2004). Given this criteria, the Apcar and Pederson stream systems are very vulnerable. Some authors have suggested that species persistence despite losses in genetic variation may be attributable to their life history strategy, though the mechanism that supports persistence despite low genetic variation is not understood (Groombridge et al. 2009; Doerner et al. 2005). Some species such as cheetahs (*Acinonyx jubatus*) have little genetic diversity yet appear to be persisting quite well while other species like greater prairie chicken (*Typanuchus cupido pinnatus*) have been negatively impacted by low genetic diversity and their recovery is attributed to genetic rescue (May 1995; Westemeier et al. 1998). Regardless of whether a species can persist with low genetic variation or not, evolutionary potential is substantially reduced with low genetic variability, and managers of endangered species may not be willing to risk losing genetic variation. The Seychelles Kestrel (*Flaco araea*) and White Tail Deer (*Odocoileus virginianus*) in Kentucky are populations that have recovered from very severe, albeit short population bottlenecks ($N_e = 8$ and $< 100$, respectively) (Groombridge et al. 2009; Doerner et al. 2005). In both cases, the authors credit habitat availability, demography,
and the species life history strategy in aiding the populations’ persistence (Groombridge et al. 2009; Doerner et al. 2005). If required habitat is available, a species life history strategy could aid in population recovery by either having high recruitment rates which would quickly add individuals to the population or by having high survival whereby long lived individuals have many opportunities to reproduce and their genetic information is not lost if they do not quickly reproduce.

\[ N_e \] estimates from microsatellite data are based upon the amount of neutral genetic diversity present in a population (Luikart et al. 2010). Neutral genetic variation has been used extensively in conservation biology, at times, as a proxy for genetic variation at coding regions such as allozyme loci that are under selection (DeWoody and Avise 2000). Although, meta-analyses have found that microsatellite variation tends to reflect allozyme variation (DeWoody and Avise 2000) others have suggested this relationship is weak (Palstra and Ruzzante 2008). Regardless of how correlated variation at the two marker systems is, the effective population size required to maintain variation at coding regions is much larger (> 500) than the \[ N_e \] to maintain neutral variation (Dowling et al. 2013). However, because quantitative genetic variation is lost at a slower rate than neutral variation, the loss of microsatellite variation may serve as a timely signal for managers to act (Palstra and Ruzzante 2008). Species persistence is dependent upon their ability to adapt to a changing environment and it is imperative to implement strategies that increase genetic diversity and \( N_e \), such as increasing gene flow via increasing connectivity of populations (Palstra and Ruzzante 2008). \( N_e \) in small isolated populations is highly influenced by genetic stochasticity. The introduction of gene flow
has been shown to quickly increase $N_e$ (Palstra and Russante 2008). Anthropogenic fragmentation has removed natural connectivity in this and many other stream systems, artificially reducing $N_e$ for aquatic organisms (Osborne et al. 2010, Chen et al. 2013). Populations with effective population sizes greater than 500 are considered to be stable enough to retain their evolutionary potential (Frankham et al. 2004). However, only $\sim 30\%$ of species considered to be stable had $N_e$s greater than or equal to 500 (Palstra and Russante 2008).

Restoring connectivity to the Warm Springs Complex would likely increase $N_e$ in each stream to the global or meta-$N_e$. Overlapping similarity in genetic information among subpopulations results in a global $N_e$ that is less than the summation of all subpopulation $N_es$ (Palstra and Ruzzante 2008; Luikart et al. 2010). However, increasing gene flow among reaches would allow for genetic information unique to each subpopulation to potentially increase genetic diversity, and therefore also increase the $N_e$ of each subpopulation. Genetic drift is a swift, powerful force in small, isolated populations so maintaining current levels of genetic variation through gene flow is crucial. If Moapa dace populations remain isolated, genetic drift will continue to reduce standing genetic variation and with it $N_e$.

$N_e/N_c$ ratio

The $N_e/N_c$ ratio is a measure that quantifies the amount of genetic variation in relation to the number of individuals in a population. The temporal variation of this parameter can be especially informative for managers that are interested in understanding the population dynamics of a particular species (Luikart et al. 2010). In very fecund
species, $N_c$ can be relatively stable if gene flow is low, mating is random, sex ratios are equal, and $N_c$ is stable and relatively large (Hartl and Clark 2007). Variation in $N_c$ is much higher in fecund species like fishes and the $N_e/N_c$ ratio can quickly decrease if $N_c$ increases. Therefore, the $N_e/N_c$ ratio is a product of the fluctuations of population size, sex-ratios, and the variation of reproductive success among individuals (Hedrick 2005; Palstra and Ruzzante 2008). For species with high fecundity, the $N_e/N_c$ ratio can rapidly change if few individuals contribute disproportionately to the next generation or if there is substantial sex ratio bias (Hedrick 2005). Meta-analysis of the $N_e/N_c$ ratio in wild populations has shown a reducing effect from fluctuating $N_e$ estimates (Palstra and Ruzzante 2008). A substantial increase in $N_c$ caused the $N_e/N_c$ ratio to decrease in Apcar between 2010 (0.61) and 2012 (0.18), despite relative gains in $N_e$ (Fig. 9). The increase in $N_e/N_c$ in Pederson (2011 = 0.15, 2012 = 0.21) can be attributed to a decrease in $N_c$ between 2011 and 2012, suggesting that the population may be genetically limited. The $N_e/N_c$ ratio of this population should be carefully monitored to avoid fixation via genetic drift and local extinction. Gene flow, either natural or artificial could increase genetic diversity in Pederson population, but this would entail the removal of barriers or manual introduction of Moapa dace from Plummer or Apcar into Pederson. If $N_c$ in Pederson is genetically limited, increasing gene flow would increase $N_c$ and $N_e$. The amount of genetic diversity given the population size is relatively stable in Plummer. The large $N_e$ in Plummer is likely closer to the contemporary global $N_e$ of the species because Plummer has more equal representation from each genotype cluster than either Pederson or Plummer (Fig. 6).
Little is known about the spawning behavior of Moapa dace despite repeated studies aimed at documenting it (Scoppettone et al. 1992). Eggs at varying stages of development in the skein of female Moapa dace and the presence of year round larvae suggest that dace spawn multiple times a year (Scoppettone et al. 1992). In addition, it is likely that Moapa dace are promiscuous broadcast spawners that lack parental investment beyond gamete production because pairs, parental care, and site preparation have never been documented (Scoppettone et al. 1992; Helfman et al. 2009). It is likely that Moapa dace exhibit group spawning like most other cyprinids, including *Gila atraria* and *Gila coerulea*, which are within the same lineage (Schonhuth et al. 2012; Pyron et al. 2013). Parentage analysis could substantially contribute to predictions about spawning behavior. If Moapa dace are promiscuous or group spawners and contributions are made by many males and females to the next generation, this behavior could lead to higher $N_e$s.

In a meta-analysis of $N_e/N_c$ ratios, Palstra and Ruzzante (2008) found species of conservation concern (endangered or threatened) had a $N_e/N_c$ ratio that was higher ($0.36 \pm 0.16$) than stable populations ($0.18 \pm 0.12$). Many endangered and threatened species have fragmented habitats, which could be contributing to increased $N_e/N_c$ ratios (Palstra and Ruzzante 2008). In species with highly fragmented habitat, such as the Rio Grande silvery minnow, $N_e$ was found to decrease as density (a surrogate for $N_c$) increased, while interconnected habitat promoted a direct correlation with $N_e$ and density in Pecos bluntnose shiner (*Notropis simus pecosensis*) (Osborne et al. 2010).

**Genetic diversity within populations**
Small population size often leads to low heterozygosity, low allelic diversity, and $F_{IS}$ values significantly different from zero (Burridge and Gold 2003). Despite the fact that Moapa dace are restricted to a fraction of their historical range, low levels of genetic diversity were not observed. In comparison to other threatened and endangered cyprinids, Moapa dace have high levels of genetic diversity and observed heterozygosity. The mean number of alleles per locus (all years combined) for Moapa dace ranged from 10 in Pederson to 13 in Plummer. Some populations of endangered cyprinids such as Owens pupfish (*Cyprinodon radiosus*), Portuguese nase (*Chondrostoma lusitanicum*), rare minnow (*Gobiocypris rarus*), and Gila topminnow (*Poeciliopsis occidentalis*) were found to have few alleles per locus, with averages of 4.66, 4.00, 5.36, and 3.80 alleles per locus, respectively (Parker et al. 1999; Sousa et al. 2008; He and Wang 2010; Dehaan et al. 2012; Finger et al. 2013). Other endangered populations of cyprinids such as Jarabugo (*Anaecypris hispanica*), Rio Grande silvery minnow (*Hybognathus amarus*) and Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*) have levels of genetic diversity similar to Moapa Dace, with ranges of 7.40-13.40, 9.30-11.10 and 11.6 alleles per locus respectively (Salgueiro et al. 2003; Martin and Wilcox 2004; Alo and Turner 2005; Dehaan et al. 2012). Similar to Moapa dace, threatened Oregon chub (*Oregonichthys crameri*) were found to have high allelic diversity (> 8 mean number of alleles per locus) in 14 out of 16 surveyed populations (DeHaan et al. 2012). Moapa dace had more alleles per locus than average for fresh water fish (7.5 ± 8.1) (DeWoody and Avise 2000). High mean numbers of alleles per locus suggest relatively little genetic variation has been recently lost. However, sixteen rare alleles that were detected in 2010 were not detected in 2012 despite an increase in sample size (2010 $n = 409$, 2012 $n =$
While it is possible that some of these alleles may still be present in the population, it is also possible that they have been lost. This finding is cause for concern because it suggests that despite a recent increase in population census size between 2010 and 2012, genetic drift may be reducing allelic diversity. Between 2010 and 2012, Plummer lost the most alleles (n = 13), followed by Apcar (n = 3), and Pederson lost one allele. The high allelic diversity found in this study is not an artifact of large sample size because new alleles are detected at a logarithmic rate and others have shown that 98% of genetic diversity can be captured in as few as 30 individuals (Finger et al. 2013; Frankel and Soulé 1981). Allelic diversity in Moapa dace is high however these values represent the allelic diversity for the entire species of Moapa dace, not a subpopulation. Populations that quickly rebound from a population bottleneck lose less allelic variation than populations that remain small (Nei et al. 1975; Peery et al. 2012). If Moapa dace Nc continues to increase, it is less likely that large amounts of allelic diversity will be lost.

Similar to other population bottleneck studies, Moapa dace have lost allelic diversity faster than heterozygosity has decreased (Cournuet and Luikart 1996; Garza and Williamson 2001; Palstra and Ruzzante 2008). This is to be expected because immediately following a bottleneck genetic drift removes rare alleles which subsequently increases heterozygosity excess (Allendorf 1986; England et al. 2003). The long term effect of a population bottleneck is a reduction in heterozygosity (Allendorf 1986; Hartl and Clark 2007), which has not yet become apparent in Moapa dace. In comparison to other endangered cyprinids expected heterozygosity of Moapa dace (0.69-0.70) was found to be similar to Rio Grande silvery minnow (0.68-0.75) and Cape Fear shiner
(0.77-0.78) (Sailant et al. 2004; DeHaan et al. 2012), and much higher than Portuguese nase (0.23-0.354) or Gila topminnow (0.075-0.281) (Parker et al. 1999; Sousa et al. 2008; Dehaan et al. 2012).

**Population structure**

Bayesian genotype clustering analyses identified three relatively distinct populations that were geographically separated by current and historic barriers (Figs. 2, 4, 5). Although the number of genotype clusters that best fit the data varied by year there were consistent differences in proportional membership within genotype clusters observed among tributaries, a pattern which is supported by pairwise F\(_{ST}\) results. Pairwise F\(_{ST}\) analysis also supports the finding that Pederson is more differentiated from Apcar and Plummer than these two tributary populations are from one another despite the fact that Pederson is in a central location in the watershed (Table 6). STRUCTURE results also show a decrease in the number of genotype clusters from 2010 to 2012 which suggests and increase in mixing among subpopulation groups across the tributaries in response to restoration activities.

The strong evidence for population genetic structure among these streams likely supports the historical pattern despite the convoluted and largely undocumented history of the area. Historically, waterways were diverted into concrete channels for agriculture or into pools for recreational use (Scoppettone et al. 1992). However, it is unclear how many times stream channels have been diverted or Moapa dace have been translocated because records are rare and exact locations, dates, and numbers are often obfuscated. The only well documented example of Moapa Dace transplant was by Scoppettone and
Burge when they transplanted an unknown number of adult dace and 150 larvae from a concrete irrigation channel near Baldwin Springs (near the South Fork) into Pederson (then “Refuge Stream”) on March 23rd, 1984 (Scoppettone and Burge 1994). Prior to transplant, Pederson had been treated with a piscicide and was reported that no other fish inhabited the area (Scoppettone and Burge 1994). Current population structure shows that Pederson is dominated by genotype clusters that are underrepresented in Apcar and Plummer suggesting dace in Pederson have retained a genetic signature from Baldwin Spring and that the population has remained genetically isolated due to contemporary barriers. The Pederson population has experienced varying degrees of isolation, including a fire and ongoing restoration since the translocation (Scoppettone et al. 1998; Galati 2011; Simons personal communication; Syzdek personal communication), all of which have likely contributed to the observed structure. Moapa dace abundance plummeted to an all-time low after a fire in June of 1994 when < 35 individuals were observed in the upper reaches of Pederson stream on MVNWR. The fire in 1994 likely contributed to the severe bottleneck and low Ne currently observed in Pederson, which may have well been exacerbated by the potential founder effects from the original translocation. Subsequent surveys in 1997 suggest the Pederson population rebounded but it is likely that changes in stream configuration, fragmentation, transplants, fire, and non-native species have all contributed to the contemporary population genetic structure observed for Moapa dace in the Warm Springs area.

When each parcel of MVNWR was acquired by USFWS, Moapa dace were reportedly absent from the headwater reaches of each tributary (Hatten et al. 2013).
Population strongholds were in the lower reaches of the Warm Springs Complex and the Muddy River and were the source populations that eventually naturally colonized the upper reaches of Apcar and Plummer on MVNWR (Scoppettone et al. 1998). At the beginning of this study in 2009, a culvert at the confluence of Plummer and Apcar was removed, allowing for possible upstream movement into Plummer. It is unknown how long this barrier had been in place but STRUCTURE results suggests that Moapa dace populations have remained differentiated throughout this study and that any historical human transplants have not obscured this genetic differentiation. Between October 2009 and September 2012, Moapa dace rarely moved between Apcar and Plummer (n = 10) an observation supported by the \( F_{ST} \) analysis which suggest that gene flow may not yet be counteracting the effects of genetic drift and loss of alleles in the Plummer tributary (Finger et al. 2013). The scarcity of Moapa dace captures in Apcar 300 meters above (n = 7) and below (n = 26) the confluence with Plummer supports the hypothesis that these tributaries continue to be somewhat isolated and suggests that this habitat was not extensively used by Moapa dace during this study. In contrast, 14,263 and 12,787 shortfin mollies were captured in these same sections above and below the Plummer-Apcar confluence over the three year study. Mosquitofish were also abundant in these areas (\( n_{\text{above}} = 4600; n_{\text{below}} = 10,322 \)). These high captures were despite active removal of all captured non-native fishes. The habitat in this area is also inundated with \textit{Vallisneria sp.}, a non-native aquatic plant that has been present in the system since the early 1980’s (Scoppettone, personal communication). \textit{Vallisneria sp.}, a macrophyte which forms dense underwater patches reduces stream flow and fundamentally changes channel characteristics from clear, high flowing water with variable substrates that are preferred
by Moapa dace. As a result, *Vallisneria* may act as a barrier and be contributing to the observed population differentiation.

**Conservation implications**

The removal of barriers can be an effective means of reconnecting isolated populations by restoring connectivity among fragmented habitat patches and subsequently leading to increased gene flow and larger \( N_e \) (Palstra and Ruzzante 2008). In the Warm Springs area many physical barriers have been removed, which represents an important first step to promoting habitat connectivity. Since the removal of the culvert barrier in 2009 Apcar and Plummer are no longer physically isolated, although the extent to which marginal habitat represents a barrier to gene flow remains unknown. In contrast, Pederson is substantially more isolated. After the completion of this study, the lower culvert on Pederson was removed and replaced with a low gradient rapid which Moapa dace should be able to ascend. However further investigation is necessary to verify this. The upper Pederson barrier present at Warm Springs Road remains impassible.

Restoring habitat connectivity is the ideal method for restoring gene flow because it is a long term solution that requires only regular genetic monitoring to quantify success versus actively managing the population through human mediated admixture. Equally important is ensuring habitat quality and habitat diversity as degraded habitat can be an effective barrier to dispersal (Neville et al. 2009; Gresswell 2011; Fluker et al. 2014). Habitat connectivity and availability has been cited as one of the most important factors for population recovery and long term viability (Turner and List 2006; Foster and Keller 2011; Whiteley et al. 2013; Fluker et al. 2014). Replacing the last remaining barrier in
Pederson with a high velocity stream segment would promote natural gene flow for the highly mobile Moapa dace while preventing upstream mobility and establishment of non-native short fin molly and mosquitofish. Restoring connectivity will allow lineages to mix at rates cohesive to the biology of a highly mobile fish and will result in reduced genetic differentiation among populations. It is likely that the strong signature of Baldwin Spring in Pederson would be reduced by restoring connectivity.

An inferior strategy would be to implement human mediated admixture. Admixture, artificial gene flow, or translocations are becoming a more common conservation strategy for small isolated populations that are highly vulnerable to the effects of low genetic variation (Bouzat et al. 2009; Gompert 2012). Admixture can be controversial because it reduces the ability of populations to adapt to local conditions (Bouzat et al. 2009) and results in unnatural population mixing. While artificial gene flow has been used extensively for the persistence of populations such as the Owen’s Valley pupfish (*Cyprinodon radiosus*) (Finger et al. 2013), Mexican wolves (*Canis lupus baileyi*) (Hedrick and Fredrickson 2010), and greater prairie chickens in Illinois (Westemeier et al. 1998), restoring ecosystem connectivity is the most beneficial long term strategy for populations like Moapa dace that have retained relatively high levels of genetic variation and thus evolutionary potential.

Quantifying neutral genetic variation has been critical for adaptive management of endangered and threatened species (Schwartz et al. 1998; Schwartz et al. 2007). For small, dynamic, short lived species, such as Moapa dace, the utility of temporal genetic monitoring is especially important because of the grave risks associated with
unrecognized losses of genetic variation (Schwartz et al. 2007). Some long lived species are able to maintain genetic variation despite environmental or demographic perturbations because these species have more opportunities to reproduce (Reed 2010; Dowling et al. 2013) or are able to persist despite low genetic variation (May 1995; Reed 2010). Hence, understanding a species genetic diversity in the context of its life history may be instrumental in guiding recovery efforts (Vähä et al. 2007; Hedrick and Fredrickson 2010; Reed 2010; Dowling et al. 2013).

Perhaps the most risky management strategy for Moapa dace would be a hands-off approach. While genetic diversity and heterozygosity were found to be high in isolated reaches, small effective population sizes should serve as a strong warning to managers. The habitat has been physically reconnected in some areas, but to date there is little genetic evidence of gene flow among tributaries and Upper Pederson remains physically isolated. The results from this study suggest continued genetic monitoring and further investigation into the impact of non-native species – both plant and fish in limiting gene flow. Genetic monitoring approaches are becoming increasingly common in conservation biology because they offer powerful insight into population dynamics, ecological relationships, and the extent of genetic resources (Schwartz et al. 2007; Dowling et al. 2013).
LITERATURE CITED


Galati, L. 2011. Warm Springs Natural Area Stewardship Plan. SNWA.


TABLE LEGENDS

Table 1. The primers and their sources used on Moapa dace samples for genetic analysis.

Table 2. Primer concentrations used in polymerase chain reactions for Moapa dace.

Table 3. Number of samples (n), number of alleles (N_A), allelic richness (R_S), inbreeding coefficient (F_IS), gene diversity (H_E) and observed heterozygosity (H_o) for each locus and population of Moapa dace captured between October 2009 and September 2012 (ALL), in 2010, 2011, and 2012.

Table 4. Number of private alleles per population in Apcar, Pederson, and Plummer tributaries in the Warm Springs area, Nevada.

Table 5. Percent of molecular variance partitioned to differences among streams (Apcar, Pederson and Plummer), populations (Upper Apcar, Lower Apcar, Upper Pederson, Lower Pederson, Upper Plummer and Lower Plummer), among individuals and within individuals for all individuals, and individuals captured in 2010, 2011, or 2012.

Table 6. Pairwise F_ST values below the diagonal comparing Moapa dace between stream reaches for all Moapa dace captured between October 2009 and September 2012.
FIGURE LEGENDS

Figure 1. Regional map depicting the current hydrology of southeastern Nevada. The study site is part of the Warm Springs area.

Figure 2. Study site for Moapa Dace is comprised of three streams; Apcar, Pederson, and Plummer. Boundaries for each stream segment are depicted in red (Apcar), blue (Pederson), and green (Plummer). Moapa Valley National Wildlife Refuge property is on the South and East side of the Warm Springs Road. Southern Nevada Water Authority property is on the North and West sides of the Warm Springs Road. Station locations are represented by white dots. Barriers to fish movement are represented by black ovals.

Figure 3. (A) The natural log of the probability of the data and (B) the delta-K method (Evanno et al. 2005) identified \( k = 2 \), 5 genotype clusters as the best fit of the data for Moapa dace captured between October 2009 and September 2012.

Figure 4. Bayesian clustering of Moapa dace identifies (A) \( k = 2 \) or (B) \( k = 5 \) genotype clusters; three distinct subpopulations.

Figure 5. Population STRUCTURE of Moapa dace captured between October 2009 and September 2012 projected onto the landscape. Proportional membership in five genotype clusters identify three subpopulations among stream reaches Apcar, Pederson and Plummer. Upper and Lower reaches of each stream segment, divided by Warm Springs Road, appear to be well mixed.

Figure 6. Average Bayesian clustering summary of ten iterations of \( k = 5 \) genotype clusters from all Moapa dace captured between October 2009 and September 2012.

Figure 7. Delta-k for \( k \) genotype clusters for Moapa dace in (A) 2010, (B) 2011, and (C) 2012. Average Bayesian clustering summary of ten iterations for all Moapa dace in Apcar, Pederson, and Plummer streams in (D) 2010 \( k = 4 \); (E) 2011 \( k = 3 \); and (F) 2012 \( k = 2 \).

Figure 8. Within tributary delta-k and STRUCTURE output for Apcar (A), Pederson (B) and Plummer (C) in 2012.

Figure 9. (A) Annual average Lincoln-Peterson population estimate (\( N_c \)), (B) genetic effective population size (\( N_e \)), and (C) \( N_e/N_c \) ratio of Moapa dace captured in Apcar, Pederson, and Plummer streams in 2010, 2011, and 2012. Bars indicate 95% confidence intervals for \( N_c \) and \( N_e \).
### Table 1.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genbank Asscession</th>
<th>PCR</th>
<th>Core motif</th>
<th>PRIMER SEQUENCE (5'-3')</th>
<th>Source</th>
<th>Genus species</th>
<th>Common name</th>
<th>Range</th>
<th>Number of Alleles</th>
<th>$H_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lce-C1</td>
<td>AY962241</td>
<td>One</td>
<td>VIC</td>
<td>F: AGGTGTTGGTTCCCTCCCG</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>142-182</td>
<td>13</td>
<td>0.86</td>
</tr>
<tr>
<td>LkB-072</td>
<td></td>
<td>(TG)13</td>
<td></td>
<td>R: TGTTATCTCGGTTCACGAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FJ601720</td>
<td></td>
<td>One</td>
<td>NED</td>
<td>F: TCATTAGGGAGGCTTTATTC</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>187-209</td>
<td>6</td>
<td>0.66</td>
</tr>
<tr>
<td>Lsou08</td>
<td></td>
<td>PCR2</td>
<td>(GT)17</td>
<td>R: CCTTCTAAACATTTTGTCAGGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF209003</td>
<td></td>
<td>One</td>
<td>FAM</td>
<td>R: TAGGAACGAAGACCTGTGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LkC-090</td>
<td></td>
<td>(TG)15; GG(TC)3</td>
<td></td>
<td>F: TCAGACCAACACTAACCGACC</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>255-297</td>
<td>20</td>
<td>0.90</td>
</tr>
<tr>
<td>FJ601722</td>
<td></td>
<td>One</td>
<td>VIC</td>
<td>R: GCGGTGTCCAGAATG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gbi-G34</td>
<td></td>
<td>PCR3</td>
<td>(GATA)14</td>
<td>F: GTTCGGGGTGCTCCAACCTCC</td>
<td>Meredith and May 2002</td>
<td>Gila bicolor obesa</td>
<td>Lahontan Tui Chub</td>
<td>250-334</td>
<td>22</td>
<td>0.91</td>
</tr>
<tr>
<td>AF393666</td>
<td></td>
<td>One</td>
<td>FAM</td>
<td>R: GTCGGCGCTGTACCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gbi-G39</td>
<td></td>
<td>PCR4</td>
<td>(GATA)11</td>
<td>F: GAGCGGGTGGATTTTTACTTTAT</td>
<td>Meredith and May 2002</td>
<td>Gila bicolor obesa</td>
<td>Lahontan Tui Chub</td>
<td>262-312</td>
<td>22</td>
<td>0.81</td>
</tr>
<tr>
<td>AF393668</td>
<td></td>
<td>Two</td>
<td>VIC</td>
<td>R: ATTTCATTACCCGGGTCAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel_223</td>
<td></td>
<td>PCR4</td>
<td>(TATC)18</td>
<td>F: CATACGATTTTTTTATTAGCTTG</td>
<td>Keeler-Foster et al. 2004</td>
<td>Gila elegans</td>
<td></td>
<td>233-265</td>
<td>22</td>
<td>0.83</td>
</tr>
<tr>
<td>AY269262</td>
<td></td>
<td>Two</td>
<td>NED</td>
<td>R: GTTACTGTGTTGTTGAGGAAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GilaD17</td>
<td></td>
<td>PCR4</td>
<td>(GT)13</td>
<td>F: TGGGACAGAAAGAGAAAAT</td>
<td>Dowling et al. 2005</td>
<td>Gila robusta</td>
<td>Roundtail Chub</td>
<td>256-282</td>
<td>9</td>
<td>0.61</td>
</tr>
<tr>
<td>N/A</td>
<td></td>
<td>Two</td>
<td>FAM</td>
<td>R: ATAAAGAGACGTTAAAGAAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL1-2b</td>
<td></td>
<td>PCR4</td>
<td>(TG)12</td>
<td>F: TTTGACTAGTAAACGACATCA</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>172-202</td>
<td>9</td>
<td>0.54</td>
</tr>
<tr>
<td>FJ468347</td>
<td></td>
<td>Two</td>
<td>FAM</td>
<td>R: CACGACAAGTTCTCAACATCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lsou05</td>
<td></td>
<td>PCR5</td>
<td>(CA)17</td>
<td>F: CTGGAAGAGACCCTTGCTCG</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>209-249</td>
<td>19</td>
<td>0.83</td>
</tr>
<tr>
<td>EF209002</td>
<td></td>
<td>Two</td>
<td>FAM</td>
<td>R: CCCACATCTGCTGACTCTGAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Florescent tag sequences on forward primers are VIC=gcggataacaatattcacaagg, NED=taaaacgacggccagtgc, FAM=tttcccagtcacgacgttg; Reverse tag=gtttctt
Table 2.

<table>
<thead>
<tr>
<th>PCR</th>
<th>Primer</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Florescent Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR1</td>
<td>LleB-072</td>
<td>0.17</td>
<td>0.50</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Lce-C1</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>PCR2</td>
<td>Lsou08</td>
<td>0.08</td>
<td>0.25</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>LleC-090</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>PCR3</td>
<td>Gbi-G34</td>
<td>0.42</td>
<td>1.25</td>
<td>0.42</td>
</tr>
<tr>
<td>PCR4</td>
<td>Gel_223</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>GilaD17</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>BL1-2b</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Gbi-G39</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>PCR5</td>
<td>Lsou05</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
</tbody>
</table>

All quantities in μM
### Table 3.

<table>
<thead>
<tr>
<th>Locus</th>
<th>APCAR</th>
<th>PEDERSON</th>
<th>PLUMMER</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>413</td>
<td>71</td>
<td>141</td>
<td>309</td>
</tr>
<tr>
<td>N_A</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>R_S</td>
<td>12.99</td>
<td>12.74</td>
<td>11.99</td>
<td>12.31</td>
</tr>
<tr>
<td>F_IS</td>
<td>-0.01</td>
<td>-0.09</td>
<td>-0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>H_E</td>
<td>0.85</td>
<td>0.88</td>
<td>0.86</td>
<td>0.85</td>
</tr>
<tr>
<td>H_O</td>
<td>0.86</td>
<td>0.96</td>
<td>0.93</td>
<td>0.84</td>
</tr>
<tr>
<td>LleB-072</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>N_A</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>R_S</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>F_IS</td>
<td>0.03</td>
<td>0.06</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>H_E</td>
<td>0.66</td>
<td>0.68</td>
<td>0.67</td>
<td>0.65</td>
</tr>
<tr>
<td>H_O</td>
<td>0.64</td>
<td>0.64</td>
<td>0.66</td>
<td>0.63</td>
</tr>
<tr>
<td>LleC-090</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>N_A</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>R_S</td>
<td>17.25</td>
<td>15.57</td>
<td>16.63</td>
<td>15.97</td>
</tr>
<tr>
<td>F_IS</td>
<td>0.03</td>
<td>-0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>H_E</td>
<td>0.90</td>
<td>0.89</td>
<td>0.89</td>
<td>0.90</td>
</tr>
<tr>
<td>H_O</td>
<td>0.87</td>
<td>0.92</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>Gbi-G34</td>
<td>22</td>
<td>19</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>N_A</td>
<td>22</td>
<td>19</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>R_S</td>
<td>20.99</td>
<td>18.18</td>
<td>18.57</td>
<td>19.84</td>
</tr>
<tr>
<td>F_IS</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td>H_E</td>
<td>0.93</td>
<td>0.93</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>H_O</td>
<td>0.96</td>
<td>0.97</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>GilaD17</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>N_A</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>R_S</td>
<td>7.91</td>
<td>6.99</td>
<td>6.62</td>
<td>7.52</td>
</tr>
<tr>
<td>F_IS</td>
<td>-0.02</td>
<td>0.09</td>
<td>0.00</td>
<td>-0.03</td>
</tr>
<tr>
<td>H_E</td>
<td>0.68</td>
<td>0.68</td>
<td>0.69</td>
<td>0.67</td>
</tr>
<tr>
<td>H_O</td>
<td>0.69</td>
<td>0.61</td>
<td>0.68</td>
<td>0.70</td>
</tr>
<tr>
<td>BL1-2b</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>N_A</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>R_S</td>
<td>12.87</td>
<td>11.61</td>
<td>8.65</td>
<td>10.91</td>
</tr>
<tr>
<td>F_IS</td>
<td>-0.04</td>
<td>0.04</td>
<td>-0.03</td>
<td>-0.06</td>
</tr>
<tr>
<td>H_E</td>
<td>0.84</td>
<td>0.85</td>
<td>0.84</td>
<td>0.83</td>
</tr>
<tr>
<td>H_O</td>
<td>0.88</td>
<td>0.82</td>
<td>0.86</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Table 4.

<table>
<thead>
<tr>
<th>Locus</th>
<th>APCAR</th>
<th>PEDERSON</th>
<th>PLUMMER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lce-C1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LleB-072</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Lsou08</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>LleC-090</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Gbi-G34</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GilaD17</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>BL1-2b</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Lsou05</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2</strong></td>
<td><strong>1</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>
Table 5.

<table>
<thead>
<tr>
<th>Source</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among three streams</td>
<td>1.2%</td>
<td>1.5%</td>
<td>1.3%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Among six populations</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.5%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Among Individuals</td>
<td>11.1%</td>
<td>6.2%</td>
<td>11.3%</td>
<td>11.9%</td>
</tr>
<tr>
<td>Within Individuals</td>
<td>87.4%</td>
<td>92.0%</td>
<td>86.9%</td>
<td>86.4%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table 6.

<table>
<thead>
<tr>
<th></th>
<th>APCAR</th>
<th>PEDERSON</th>
<th>PLUMMER</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>413</td>
<td>250</td>
<td>894</td>
</tr>
<tr>
<td>APCAR</td>
<td>-</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>PEDERSON</td>
<td>0.0204</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>PLUMMER</td>
<td>0.0096</td>
<td>0.0252</td>
<td>-</td>
</tr>
</tbody>
</table>

*Signifies populations are significantly different. P-value was obtained after 60 permutations

*Indicative adjusted nominal level of 5% for multiple comparisons is 0.016667
Figure 2.
Figure 3.
A. $k = 2$

B. $k = 5$

Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.

A. \( n = 309 \)

\[ \Delta k \]

\( n = 309 \)

UPPER APCAR  LOWER APCAR

B. \( n = 143 \)

\[ \Delta k \]

\( n = 143 \)

UPPER PEDERSON  LOWER PEDERSON

C. \( n = 413 \)

\[ \Delta k \]

\( n = 413 \)

UPPER PLUMMER  LOWER PLUMMER
Figure 9.
CHAPTER 2: POPULATION DYNAMICS OF MOAPA DACE

INTRODUCTION

Some of the most informative parameters of population regulation are also the most complicated and difficult to derive. For many animal populations, accurately estimating abundance is an arduous or impossible task, so the rate of population change is estimated instead of abundance. Lambda, the rate of population change, can be directly derived from survival and recruitment estimates. Estimating survival and recruitment requires robust data sets but the value of these estimates is exceptionally high, especially to managers that are charged with protecting and restoring endangered species to historic levels. Managers are specifically interested in understanding the relative contribution of survival and recruitment on lambda because these parameters describe how a population is regulated over space and time. In addition, deriving these estimates is essential for quantifying the relative contribution of recruitment and survival because they might identify factors limiting population growth and species recovery.

The Endangered Species Act (ESA) was designed to provide special protection for listed species and their ecosystems (USFWS 1994, Himes Boor 2014). To remove a species from the endangered species list, all specifications on the individual species’ recovery plan must be met (USFWS 1994; Himes Boor 2014). Typically these include minimum population sizes over a specified duration and of various age classes, the protection and occupation of historic habitat, and the removal of imminent threats such as those caused by non-native species, humans, parasites, and disease (USFWS 1994; Himes Boor 2014). Due to the sensitivity of such species, it is imperative for managers of
endangered species to accurately and precisely understand the rate of population growth and how survival and recruitment parameters influence population dynamics (Williams et al. 2002). Individual survival and recruitment directly reflect individual fitness which may vary with age, body size, sex, genotype, population, and abiotic factors (Lebreton et al. 1992). By gaining insight into how these life history parameters vary among groups and over time, managers can better interpret observed trends and adapt management practices to best serve population recovery (Williams et al. 2002). Information theoretic approaches have been used to estimate vital rates using various model frameworks such as Cormack-Jolly-Seber (CJS) models (Williams et al. 2002). Various competing hypotheses or generalized linear models are assessed in a Pradel model using maximum likelihood which ranks models based on the observed data (Williams et al. 2002).

Capture–mark–recapture (CMR) studies of freshwater fish populations are rare because low detection probabilities limit inference about population dynamics (Pine et al. 2003). In addition, fisheries CMR studies have difficulties estimating tag retention rates and there is often uncertainty surrounding how representative the sample is of the whole population (Hewitt et al. 2010; Pine et al. 2003). Population dynamics studies can also be costly and logistically difficult to implement however, careful study design and tag-retention pilot studies can solve these problems (Hewitt et al. 2010). For fish populations, the utilization of remote sensing technology has increased detection rates and the ease of accumulating robust, informative data sets (Hewitt et al. 2010). Molecular techniques have become more affordable and easy to use for individual identification in lieu of a physical tag, and these methods are common in population dynamics studies when
capturing individuals is logistically difficult or costly (e.g. grizzly bears, black bears, caribou) (Boulanger et al. 2002; Peacock et al. 2011; Hettinga et al. 2012), or usage of tags is logistically challenging due to the body size of the study organism, such as in this study. Accumulating these data sets is challenging for all animal populations, but CMR studies are especially rare for fish populations. However, these methods are becoming more common in fisheries studies because fish biologists are realizing that CMR methods are a powerful tool for understanding how populations change over space and time. If these studies are carefully designed it is possible to identify biotic and abiotic factors that are affecting survival and recruitment in a population. Many fishes are endangered because little is known about population regulation. It is exceptionally difficult to effectively manage populations without understanding population demographics; survival, recruitment and the rate of population change. To assist managers who are charged with making crucial decisions regarding prioritizing conservation efforts for Moapa dace and the Warm Springs area, the goals of this study were to: i) estimate bimonthly ($\phi_b$) and annual ($\phi_a$) survival of Moapa dace, ii) estimate whether survival varies with size (fork length) or heterozygosity, iii) estimate recruitment ($f$) of Moapa dace, iv) estimate the annual rate of population growth ($\lambda$), and v) describe what factors may be causing the observed trends.

METHODS

Description of study area

Geothermal springs and small rivers are the last remnant freshwater ecosystems of the ancient Pleistocene rivers and lakes that once extensively covered much of what is
now southern Nevada and southern California (Reheis 1999; Reheis et al. 2002; Smith et al. 2002). Since the recession of these large rivers and lakes, several unique lineages of freshwater fishes have adapted to live in these small and stressful remnant environments (Hubbs and Miller 1948a; Smith et al. 2002; Martin et al. 2004). Low dissolved oxygen levels, warm water (27 - 32˚C), and relatively high flows have shaped the unique physiology, biology, and life history of Moapa dace (*Moapa coriacea*), a small bodied (<120 mm) cyprinid endemic to the Muddy River and its tributaries in Clark County, Nevada (Figs.1, 2) (Hubbs and Miller 1948b; Scoppettone et al. 1992). Moapa dace are strong swimmers that often congregate in large feeding groups, capturing invertebrates and plant items that drift downstream in the water column (Scoppettone et al. 1992). This high energetic feeding strategy is similar to that observed in trout species (Gunckel et al. 2002). Early surveys of Moapa dace found larger individuals in the Muddy River than in the warmer headwater tributaries (Scoppettone et al. 1992). Similar to other fishes, female Moapa dace produce more eggs at larger body sizes (Scoppettone et al. 1992; Closs et al. 2013) and reproduction occurs year round but is highest in the spring (Scoppettone et al. 1992). Moapa dace are short lived fish, typically living less than five years (Scoppettone et al. 1992). Listed under the ESA as endangered in 1967 (USFWS 1995), anthropogenic habitat alteration, range restriction, habitat fragmentation, and non-native species have all contributed to Moapa dace population declines (Scoppettone et al. 1992; Scoppettone et al. 2005; Hatten et al. 2013). Historically, water at spring sources was diverted for agriculture or pooled and chlorinated for recreation (Scoppettone et al. 1992). More contemporary habitat threats include dams, barriers, ground water pumping, and non-native species (Scoppettone et al. 2005; Hatten et al. 2013). Snorkel surveys first
conducted in 1984 documented Moapa dace abundance at approximately 2,800 individuals (Scoppettone et al. 1992). The highest population estimate was 3,841 adult Moapa dace in 1994 in the Muddy River and its tributaries (Scoppettone et al. 2005). In 1995, the population experienced a population decline when a diversion dam was removed and the non-native predator blue tilapia (*Oreochromis aurea*) invaded, reducing abundance to 1,565 individuals in 1997, and to less than 1,000 individuals in 1999 and 2000 (Scoppettone et al. 2005). A natural barrier was enhanced to exclude blue tilapia from part of the Warm Springs area and later a gabion barrier was installed to prevent further invasion into the Apcar, Pederson, and Plummer tributaries (Scoppettone et al. 2005). Currently, Moapa dace are restricted to 2.8 kilometers of stream habitat in these tributaries representing only 10% of their historical range (Fig. 2). Moapa dace abundance was lowest in 2008 and 2009 when four consecutive snorkel surveys observed approximately 500 Moapa dace (Simons, unpublished data). More recent snorkel surveys have estimated the population to be around 1,600 individuals (Simons, unpublished data). The long term goal is to delist Moapa dace which requires that at least 75% of historical habitat is available for different life stages, that the adult population is greater than 6,000 individuals for five consecutive years and that threats from non-native fishes, and parasites do not impact the long term survival of this species (USFWS 1995). Biannual snorkel surveys have been crucial for monitoring the population size of Moapa dace but additional information about how survival and recruitment parameters contribute to population growth are needed to better understand the observed population fluctuations.

**Field Methods**
Apcar, Pederson, and Plummer tributaries were sampled bimonthly for Moapa dace beginning October 2009 and ending September 2012 for a total of 18 sampling periods. Gee minnow traps with quarter inch mesh were set twice per week long sampling occasion in a robust design sampling scheme. I assumed the population was closed between trapping sessions within each bimonthly trapping session. I refer to bimonthly trapping sessions as primary encounter occasions and trapping sessions within bimonthly sampling occasions as secondary encounter occasions. Traps were set every five meters on Moapa Valley National Wildlife Refuge (MVNWR) property in Plummer and Apcar tributaries, and every 25 meters throughout the rest of the study area at a total of 163 sampling locations, hereafter stations (Fig. 2). The initial goal of this study was to capture as many Moapa dace as possible, so traps were set at greater frequency in areas believed to have more Moapa dace in an attempt to increase capture probability. All traps were baited with dog food and set for three to 28 hours. Traps set in more stressful microclimates such as warm water temperatures or high velocities were pulled after three to eight hours. Most traps (90.6%) were deployed for less than 25 hours ($n = 7008, \bar{x} = 14.14$ h, $SD = 8.5$, median = 13.36). Intervals between secondary captures ranged from one minute to 96 hours; 86% of closed capture periods were less than 50 hours. There were a few occasions when all traps were not set. After the first two sampling occasions, sampling design was modified from low trap density (every 25 meters) to high (every five meters) trap densities in Apcar and Plummer in MVNWR. On July 1, 2010, a fire burned through the Warm Springs Natural Area on Southern Nevada Water Authority Property and 84 of the 169 stations could not be sampled during July 2010 due to hazardous working conditions. The following sampling period, September 2010, 15% of
stations could not be accessed due to ongoing palm tree removal. Finally, restoration work conducted by Southern Nevada Water Authority prevented some traps \((n_i)\) from being set in July 2011 \((n_i = 4)\), September 2011\((n_i = 2)\) and January 2012 \((n_i = 6)\). I account for variation in trapping effort by allowing for full temporal spatial variation in capture probabilities (see below).

I measured all captured Moapa dace to fork length and took a small tissue sample approximately 2 mm\(^2\) (hereafter, fin clip) from the top caudal fin on the first secondary capture occasion and from the bottom caudal fin on the second secondary capture occasion. Fin clips collected between October 2009 and March 2011 were stored in one ml of 95% ethanol; after May 2011 protocol was modified to increase yields of DNA and fin clips were dried and stored in wax paper.

Beginning in March 2011, I began a pilot study where I tested the use of UNO PICO-ID ISO Transponder tags (hereafter tag) from UNO Roestvaststaal BV in a subsample of Moapa Dace. Throughout the study, 74 individuals were anesthetized using Tricaine mesylate (MS-222) and a 7mm x 1.25 mm tag was injected into the body cavity between the pyloric caeca and the pelvic girdle using a sterile syringe. I deployed tags in groups of 10 - 15 in Upper Plummer and Upper Apcar until November 2011. Only Moapa dace healthy in appearance, larger than 49 mm fork length, and weighing more than one gram were considered for tagging. All Moapa dace greater than 49 millimeters and not isolated by barriers were scanned for the presence of a tag.

**Genetic Analysis**
To identify individuals, I used ten polymorphic microsatellite loci developed for closely related species (Table 1) to create a genetic tag for each individual. Ethanol was evaporated from those fin clips stored in ethanol and then DNA was extracted from all samples using QIAGEN DNeasy 96 tissue extraction kits according to the manufacturer’s instructions for animal tissues (QIAGEN 2006). I modified this protocol to increase yields of DNA by eluting the DNA in two steps. In each elution step I added 75 µl of 55°C Buffer AE to each sample, incubated samples at 22°C for five minutes, then centrifuged samples for two minutes (QIAGEN 2006). DNA yields were quantified for each sample on a Labsystems Fluoroskan Ascent fluorometer (Nevada Genomics Center, http://www.ag.unr.edu/genomics/). I dried down replicate samples of DNA (30-70 ng) for five multiplexed polymerase chain reactions (PCR). I conducted all five PCRs on TECHME ® TOUCHGENE THERMAL CYCLER machines. Microsatellite loci were amplified and florescent labels VIC, NED, or FAM were attached to each product. I optimized microsatellite loci for use with Moapa dace using known primers from Eurasian dace (Leuciscus leuciscus, n = 6), Lahontan Tui Chub (Gila bicolor n = 2), Roundtail Chub (Gila robusta, n = 1), and Bonytail Chub (Gila elegans n = 1) (Dubut et al. 2009, Meredith and May 2002, Keeler-Foster et al. 2004, Dowling et al. 2005). Primer details are described in Table 1.

I diluted and optimized PCR products in two 96 well panels then analyzed fragments on a Perkin Elmer Applied Biosystems 3730 Genetic Analyzer at the Nevada Genomics Center (http://www.ag.unr.edu/genomics/). I used GeneMapper Software (version 3.7) to genotype alleles at each locus. All samples were analyzed at ten
microsatellite loci (Table 1) by two independent observers. A minimum of five di-allelic loci (ten measured fragments) were required for individual identification. I identified individuals using GENECAP, an excel macro that calculates the probability of two individuals within a population sharing the same genotype (probability of identity) under Hardy Weinberg Equilibrium $HW P(ID)$ and the probability of being siblings $SIB P(ID)$, a more conservative measure (Wilberg and Dreher 2004). GENECAP also identifies exact genotype matches among samples, lists all genotypes that mis-match by one or two alleles, and creates capture histories for each individual genotype (Wilberg and Dreher 2004). I re-evaluated or removed all genotypes with one or two mismatched alleles or with high $SIB P(ID)$, or high $HW P(ID)$ values ($P > 0.05$). Tissue samples that resulted in low DNA yields and failed to amplify at enough loci were not reanalyzed. I did this to efficiently, accurately, and economically process the large volume of samples.

Microchecker (version 2.2.3, Van Oosterhout et al. 2004) was used to test for allelic dropout and presence of null alleles. FSTAT (version 2.9.3, Goudet 2002) was used to calculate expected heterozygosity, observed heterozygosity, and allelic richness within each population (Table 1).

**Data Analysis**

I created individual capture histories using genetic tags and to ensure consistent mark-recapture sampling I did not use captures from transponder tags to create capture histories. The effect of the transponder tag was quantified in detection and survival estimates by including tag (TAG) as a binomial time varying covariate. Once an individual was tagged, it remained in that group, regardless of whether or not the tag was
detected. This design assumed that any effect associated with the tag was persistent. I combined secondary capture occasions within each primary occasion to create 18 open capture occasions. This increased encounter probability and eliminated immigration and emigration parameters associated with the robust design framework. Immigration for this system is zero because a downstream gabion barrier (Fig. 2) prevents individuals from entering the population and emigration is expected to be essentially zero. Over the three year period, Moapa dace captures were rare (n = 10) in the most downstream area of the study site, 200 meters above the gabion barrier. Based on the criteria that the system is effectively closed and that I had no mortality associated with capture, I assumed apparent survival to be true survival. In support of the closure assumption, biannual snorkel surveys of the Muddy River conducted by the Muddy River Biological Advisory Committee (MRBAC) identified few Moapa dace outside the study area in February 2010 (n = 5) and one individual in subsequent surveys in August 2011 and February 2012. In this study, I defined recruitment as when a dace becomes a trappable size, typically 30 - 50 mm, though trappable size varied among individuals. I defined each year as 2010: October 2009 – October 2010, 2011: November 2010 – October 2011, and 2012: November 2011 - September 2012. I divided the six sampling occasions within each year into two seasonal trends that had three seasons each; the first (sea) was September to December, January to April, and May to August, the second (sea2) was November to February, March to June, and July to October.

I used Pradel models in program MARK (White and Burnham 1999) to estimate bimonthly capture probability (p), survival (\( \phi_b \)), recruitment (f), and rate of population
growth ($\lambda$) of Moapa dace over the three year period. Pradel models use reverse time capture histories to estimate entry rate into a population (recruitment). In this analysis, I estimated primary capture probability ($n = 18$), survival between each sampling period ($n = 17$), and recruitment between each sampling period ($n = 17$) using a Pradel Survival and Recruitment model. I estimated the rate of population growth using a Pradel Survival and Lambda model. The assumptions associated with Pradel models are: 1) sampling is instantaneous, 2) individual tags are not lost or missed, 3) capture probability among individuals is equal, 4) tagging does not impact future capture probability or survival, 5) emigration from study site is permanent, 6) individual fates regarding capture and survival probability are independent, 7) all individuals belong to a single age class, 8) new individuals do not enter the population because sampling methods improve and 9) the size of the study site is constant (Pradel 1996; Williams et al. 2002).

**Individual and group covariates**

I assigned all captured individuals to a population based on where they were captured most often or in the case of a tie to the last capture location, although very few individuals moved among the six stream segments (Fig 2). I categorized population groupings based upon i) stream, ii) property boundaries (also separated by Warm Springs Road), iii) remnant fish barriers, and iv) general habitat groupings that vary among stream segments and were temporally dynamic. Population designations are depicted in Figure 2. I standardized fork length ($\overline{x} = 0.0, SD = 1.0$) and included it as a time varying covariate. I calculated and standardized heterozygosity ($\overline{x} = 0.0, SD = 1.0$) for each individual based on the most complete genotype available for that individual at $n \leq 8$ or $n$
≤ 10 loci. Two loci with null alleles were included in heterozygosity calculations at n ≤ 10 loci but were excluded in n ≤ 8 loci. Of the dace I tagged with transponder tags, I imposed the effect of the tag upon application and for every capture occasion thereafter as a binomial time varying constant. I included the tag covariate in candidate models of both survival and encounter probability. I report estimates of demographic parameters from non-tagged fish because the presence of transponder tags influenced these parameters (see below).

Model creation and selection

Model selection occurred in four stages. In the first three stages I used the Pradel survival and recruitment model in Program MARK to estimate encounter probability (stage one), survival (stage two), and recruitment (stage three). First, I modeled encounter probability with a constant (dot) structure on each survival and recruitment. Second, I used the most parsimonious encounter probability model and a constant recruitment structure to model survival. Third, I used the structure from the most parsimonious encounter probability and survival models to model recruitment. In the fourth stage I used the group and time structure from the most parsimonious encounter probability and survival models to estimate lambda using the Pradel survival and lambda model in Program MARK. Within each stage, I tested all a priori time (n = 8), group (n = 3), and biologically applicable individual covariate (n = 0 - 5) models against full interaction (g * t), additive (g + t), and constant (. ) models (Table 2). To account for variance in encounter probability, I also tested three sample variance models. Two of these sample variance models accounted for variance in field sampling methods; the number of traps set (TN) and number of trap hours (TH). Both TN and TH varied by population and
sampling period. I also tested a model that accounted for variance in lab methods. Low DNA yields or failed PCR products made it impossible for me to identify some individuals so I calculated the probability of genotyping an individual; \( PG = p \) (successful genotype | captured ) for each population and each sampling occasion. To limit the number of models tested, I combined the most parsimonious group model with the most parsimonious time model in an additive model that was tested against all previously mentioned models (Table 2). Next, I added each sample variance parameters or individual covariates to the most parsimonious group plus time model; (e.g. encounter probability \( g + t + TN \), \( g + t + PG \), \( g + t + TAG \)). Finally, I tested a model that incorporated individual covariates (including field variance and lab variance in encounter probability models) into the most parsimonious group and time models e.g. \( p(g + t + TN + PG + TAG) \). Sample variance parameters were only tested in encounter probability models. The biologically applicable individual covariates that I tested in encounter probability models were tag presence (TAG), fork length (FL), and fork length squared (\( FL^2 \)). In survival models I tested the covariates FL, \( FL^2 \), TAG, heterozyosity at \( \leq \) eight loci (H8), and heterozygosity at \( \leq \) ten loci (H10). No covariates were tested in recruitment models. I tested two additional group structure models (g.A and g.P) in survival and recruitment model sets when group betas were not estimable for the smallest population (Lower Pederson). This tested two hypotheses for each survival and recruitment model; was survival (or recruitment) in Lower Pederson (the smallest population) more similar to the population upstream (Upper Pederson, g.P), or was survival (or recruitment) in Lower Pederson more similar to the population downstream (Lower Apcar, g.A)? In the fourth stage, models including FL and fork length \( FL^2 \) did not
converge in the Pradel survival and lambda model framework, so they were removed along with heterozygosity from the estimation of lambda. Only non-tagged individuals were used to estimate rate of population growth. Annual lambda estimates for 2011 and 2012 were calculated as the product of bimonthly lambda estimates from November 2010 to September 2011, and November 2011 to September 2012, respectively. While the model framework lent itself to extrapolate one additional lambda estimate to derive three complete annual lambda estimates for 2010, 2011, and 2012, I was concerned that lambda estimates from the first few sampling periods would be biased high because of false negatives associated with insufficient DNA yields and PCR failure. Lambda is particularly sensitive to violations associated with false negatives so I only calculated lambda for 2011 and 2012.

I used an information theoretic approach for model selection (Burnham and Anderson 2002). I first adjusted for overdispersion (c) using goodness of fit tests 2 and 3 in Program RELEASE on the model with the most estimable parameters as suggested by Burnham and Anderson (2002) and used quasilikelihood to evaluate models. All models that failed to converge were removed from the analysis and model rankings. I derived 95% confidence intervals by taking the standard deviation of 500 bootstrapped annual estimates that I generated from bimonthly estimates and standard errors of each population and year. Confidence intervals for individual covariates were quantified using the delta method.

RESULTS
Between October 2009 and September 2012, I captured and genetically identified 1,557 individual Moapa dace. I estimated overdispersion to be $\hat{c}=1.056905$ ($\chi^2 = 313.9007$, 297 df, $p = 0.2395$) and this was used to adjust AIC$_c$ to quasi-AIC$_c$ (QAIC$_c$). After removing within sampling period recaptures, I captured a total of 3,002 independent captures and had an average of 1.93 captures per individual. I captured and identified 68.8% of individuals in two or more sampling periods. Mean fork length was $50.7 \pm 11.2$ SD mm (range 19 - 93 mm). Less than 18% of captures were smaller than 41 mm, the smallest known size that Moapa dace are reproductive. Of the 74 Moapa dace that were tagged, 69 were included in this analysis. Failure of amplification or inconsistent amplification of both alleles at a minimum of five loci resulted in the removal of five fish tagged with transponders. Average heterozygosity at $\leq$ eight loci was $0.688 \pm 0.15$ (range 0 - 1). Average heterozygosity was $0.635 \pm 0.14$ (range 0.125 - 1.0) at $\leq$ ten loci. Of the Moapa dace that were captured and clipped, 88.6% were successfully identified. The majority of failed identifications occurred in the first seven sampling occasions. Low identification rates in January 2010, March 2010, and September 2010 were due to extremely low DNA yields in many samples.

The most general encounter probability model contained 108 parameters, the most general survival, recruitment, and population growth models each contained 102 parameters (Table 2). All interactive models failed to converge, so only additive group and time models were considered in further analyses. Encounter probability of Moapa dace was low in 2010, but relatively higher in 2011 and 2012 (Fig. 3). Low encounter probabilities are a common limitation in capture-recapture studies of fish populations.
(Pine et al. 2003). In this study, I used intensive bimonthly sampling as a means of increasing capture probability and power. The most parsimonious capture probability model included additive variation among populations across sampling occasions and sampling effort covariate (TN), the probability of successfully genotyping individuals post capture (PG), and whether or not the dace was tagged (TAG). This most parsimonious detection model was the only supported model of encounter probability and was used in all further analyses. Effect of TAG variance \( \beta = 1.421 \pm 0.165 \) and probability of successfully genotyping \( \beta = 1.300 \pm 0.315 \) accounted for most of the variation in encounter probability while the number of traps set accounted for less variance \( \beta = 0.008 \pm 0.0042 \). The second most parsimonious model of encounter probability was nested within the top model and included identical parameters but excluded the number of traps and probability of identification (Table 3). This second ranked model had a \( \Delta QAIC_c \) of 48.6 and was not competitive.

Encounter probability was higher in November and January than in May and July (Fig. 3). During most sampling occasions, encounter probability was highest in Upper Apcar and Upper Plummer where traps were set five meters apart and lowest in Lower Pederson, the most isolated river section (Fig. 2, Fig. 3). There were three extraction instances in the first seven sampling occasions where DNA yields from fin clips were extremely low, which resulted in PCR failure and low percentages of successful genotypes (PG). Each of these instances had up to 96 individual samples and these low DNA yields substantially reduced encounter probability. Of the sampling periods where
transponder tags were deployed, tagged dace had detection rates 2.04 times greater than non-tagged fish within the same reach.

**Survival**

The most parsimonious survival model included additive variation across years, seasons, and among five populations where Lower Pederson and Lower Apcar were pooled as one population, individual covariates FL², heterozygosity at ≤ ten loci, and whether or not a dace was tagged or not (TAG). The top survival model was well supported (w_i = 0.715). The second ranked model (w_i = 0.28) was the same as the best model except it lacked the heterozygosity at ≤ ten loci. In the top model heterozygosity at ≤ ten loci had a weak positive effect on bimonthly survival (β = 0.07 ± 0.034) and while the second ranked model was considered competitive, I used the top survival model, recognizing that inference power associated with the heterozygosity parameter is limited (Fig. 5). Heterozygosity at eight loci was not found to improve the model fit because it performed worse than the constant model (Table 3). There was a clear seasonal trend in bimonthly survival, which was highest from January to August and lowest from September to December (Fig. 4). I observed a quadratic relationship between survival and fork length (FL β = 0.395 ± 0.08, FL² β = -0.55 ± 0.04). Bimonthly survival was highest for 54 - 55 mm dace (0.913 ± 0.02; Fig 5) and ≤ 0.50 for dace smaller than 32 mm and larger than 78 mm. Moapa dace entering a reproductive size class (41 mm) had an average bimonthly survival 0.829 ± 0.03. Dace in Upper Apcar had the highest and Lower Plummer had the lowest annual survivals (Fig.7). Annual survival was lowest in 2010 (̅x = 0.27; 95% CI = 0.15 - 0.39 to 0.50; 0.38 - 0.63) and highest in 2012 (̅x = 0.47;
0.38 - 0.57 to 0.73; 0.65 - 0.80) (Fig. 7). Increases in survival in all populations were significantly different between 2010 and 2012. Over the three year study, annual survival increased by 0.23 in Upper Apcar, 0.28 in Lower Apcar, and Lower Pederson, and 0.29 in Upper Plummer, Upper Pederson, and Lower Plummer (Fig 7). While few individuals were tagged in each population (Upper Plummer; n = 53, Upper Apcar; n = 16), tags had a strong negative effect on survival (β = -1.03 ± 0.20). Tagged dace had lower bimonthly survival than non-tagged dace by 0.06 - 0.13 in Upper Apcar and 0.10 - 0.19 in Upper Plummer over the six and ten month durations when tags were deployed (Fig. 6).

**Recruitment**

The most parsimonious recruitment model had additive variation across seasons and among five groups, where Lower Pederson was pooled with Lower Apcar and carried all (wij = 0.99988) model weight (Table 3). The second ranked model, which lacked temporal variation, had a ΔQAICc = 18.84 so it was not considered competitive. Bimonthly recruitment was found to be highest in April and June, but occurred year round. Models including variation among years failed to converge, resulting in seasonal time variation only. Recruitment varied among populations and was highest in Lower Apcar and Lower Pederson, and lowest in Upper Plummer (Fig. 7). The lowest bimonthly recruitment was found to be 0.06 - 0.13 new dace > 20 mm dace per capita, while the highest bimonthly recruitment ranged from 0.20 - 0.48 (Fig. 7).
The most parsimonious model of rate of population growth model varied by year, month, and population and carried all ($w_i = 0.99954$) of the model weight (Table 4). The second ranked model, which was not competitive, ($\Delta$AICc = 16.16), excluded the year effect. The rate of population growth in 2011 was 1.53 (95% CI: 0.62 - 2.43) in Lower Apcar and Lower Pederson and 1.31 (0.54 – 2.08) in Upper Apcar. Upper Pederson (0.87; 0.37 - 1.36), Upper Plummer (0.64; 0.26 – 1.03) and Lower Plummer (0.90, 0.32 – 1.49) all experienced substantial population declines in 2011 (Fig. 9). In 2012, the rate of population growth was much greater for all populations; Lower Apcar and Lower Pederson (2.39, 0.97 – 3.80) and Upper Apcar (2.05; 0.63 – 3.46) experienced the highest rates of population growth, Upper Pederson (1.36; 0.52 – 2.19) and Lower Plummer (1.41; 0.59 – 2.23) had moderate rates of population growth, while population growth was stagnant in Upper Plummer (1.01; 0.32 – 1.49) (Fig. 9).

DISCUSSION

I found temporal and spatial variation in survival and recruitment of Moapa dace. Temporally, survival varied seasonally and increased over the three year study. Recruitment varied seasonally but did not vary or increase among years. Because the rate of population growth is directly derived from survival and recruitment, the observed increases in lambda are due to increases in survival. This is particularly interesting because increases in population growth are usually attributed to increases in recruitment. Instead, I observed stagnant annual recruitment and increases in annual survivorship among all populations. This trend suggests that some factor is limiting recruitment in the study area. Before Moapa dace were confined to their current distribution, adult dace
lived primarily in the Muddy River and migrated into the upper tributaries to spawn (Scoppettone et al. 1992). All dace in the study area were restricted to the upper tributaries of Apcar, Pederson, and Plummer. Dace recruitment may be limited because larvae and juvenile dace are competing with adult conspecifics. Throughout the study restoration increased the amount of available habitat, (see below) but restoration did not diversify habitat. The large river habitat is not essential for dace survival, but it may be crucial for future increases in population size because the river could alleviate intraspecific competition among different age classes. Removing the downstream gabion barrier would make river habitat available for adult Moapa dace. I did not observe spawning migration patterns or adult dace using downstream habitat because this habitat is degraded and this section does not mimic historic conditions of the Muddy River. The downstream gabion reduced water velocities and great densities of non-native species including plant species, *Vallisneria* sp., and fishes, short fin mollies (*Poecilia mexicana*) and mosquitofish (*Gambusia affinis*) are found in this area. If habitat remains limited the study area and intraspecific competition is limiting Moapa dace recruitment, it is likely that the population will reach the carrying capacity of these tributaries, and lambda will stabilize. Spatially I found survival contributed more to population growth than recruitment, though the relative contribution of survival and recruitment varied among populations. For example, in comparison to other populations, Lower Apcar and Lower Pederson had intermediate survival and high recruitment which resulted in the largest increases of lambda (2011 $\lambda = 1.53$, 2012 $\lambda = 2.39$). In contrast, the large increases in lambda in Upper Apcar (2011 $\lambda = 1.31$, 2012 $\lambda = 2.05$) were due to high survival and intermediate recruitment in comparison to other populations. The differences in relative
contribution of survival and recruitment to lambda among populations could be attributed to various stages of restoration. The July 2010 fire provided a unique opportunity to expedite restoration of Lower Apcar and the changes that occurred in this stream section resulted in high recruitment. I attribute the high survival in Upper Apcar to Moapa dace successfully establishing in this stream section where restoration was completed in 2009 (Hatten 2013). This finding suggests that the habitat changes associated with restoration could initially cause increases in dace recruitment, and over time, as the population establishes, this could switch to increases in survival. While the restoration hypothesis may describe the relative contribution of recruitment and survival in Upper and Lower Apcar, it doesn’t explain the observed decrease in lambda in Upper Plummer. Restoration was completed in 2006 and 2007 in Upper Plummer and despite this population having highest abundances at the beginning of this study, recruitment was low and survival was moderate in this stream section. Lambda in Upper Plummer decreased in 2011 ($\lambda = 0.64$) and was stable in 2012 ($\lambda = 1.008$). I attribute the 2011 decline in Upper Plummer to intense intraspecific competition among life stages. Upper Pederson was restored in the mid 1980’s (Hatten 2013). During the study, this population was isolated from all others by two downstream barriers. I found survival and recruitment to be intermediate in Upper Pederson, which resulted in a population decline in 2011 ($\lambda = 0.865$), and an increase in lambda in 2012 ($\lambda = 1.35$). Dramatic fluctuations could decrease genetic diversity and evolutionary potential. Expediting the removal of these barriers would allow dace to naturally utilize the habitat, and could reduce the intensity of population fluctuations. Lower Plummer had the lowest survival of all populations, though recruitment rates were intermediate. The dace population in Lower Plummer declined in 2011 ($\lambda = 0.90$) but
increased in 2012 ($\lambda = 1.41$). In comparison to other stream sections, the relative
difference in survival decreased, meaning Lower Plummer had the most dramatic
increases in survival over the three year study. Southern Nevada Water Authority
removed a large number of the fan palms from this section after the July 2010 fire, which
could have influenced these changes. I found that the largest population increases in areas
that had recently been restored. I attribute the overall increases in survival to the meta-
population recovering from observed lows ($n_{	ext{snorkel}} \geq 500$) in 2008 and 2009, and to
restoration, which increased the amount of available habitat. Natural population
fluctuations are common in fish species (Wootton 1990; Ayllon et al. 2012) and it is
likely that the carrying capacity for Moapa dace in Apcar, Pederson, and Plummer
tributaries has yet to be realized but once the carrying capacity is reached, lambda will
stabilize.

Prior to this study, no robust analyses had been done to describe the demographic
fluctuations of Moapa dace. This study provides a unique opportunity to describe the
biology of Moapa dace, throughout space and time. I found that survival of Moapa dace
varied spatially and temporally and was likely influenced by prey availability. Bimonthly
survival was lowest between October and December, and highest from February and
September. Variation in seasonal survival is common in fishes (Mitro and Zale 2002;
Carlson and Letcher 2003; Berger and Gresswell 2009). Some studies have correlated
seasonal survival variation to changes in water temperature (Martins et al. 2011; Weber et
al. 2011). Water temperatures at the spring sources are relatively constant (30-32°C) year
round in the Warm Springs area but temperature does vary seasonally in the lower stream
sections. It is unlikely that water temperature fluctuations are directly contributing to the reduced survival in October and December because the seasonal reduction in survival is not substantially greater in lower stream sections where temperature fluctuations are greatest (Fig. 4). Additionally, it is unlikely that warm or cool temperature extremes are reducing survival. If warm temperatures were responsible for reduced survival, I would observe survival to be lowest from June to September, when air temperatures are high and water temperatures stay between 30 – 32 °C throughout the study area. It is also unlikely that low dace survival is due to low water temperatures because survival is high in February before water temperatures in the lowest stream sections increase. Furthermore, Moapa dace have been documented at a range of temperatures (19 – 32 °C) much greater than those present in this study area (Deacon and Bradley 1972). Seasonal variation in survival is more consistent with the hypothesis that a reduction in the abundance of invertebrates, the primary prey of Moapa dace (Scoppettone et al 1992), may occur from September to December when survival is lowest and therefore contribute to declines in survivorship. Seasonal fluctuations in aquatic invertebrate abundance are typical and such variation in food resources have been shown to reduce growth rates and fitness in many fish species (Wootton 1990; Dineen et al. 2007; Rundio and Lindley 2011). Aquatic invertebrate diversity and abundance varies throughout the length of a stream. Typically the upper stream segments near the spring sources have less aquatic invertebrates because physical and biological resources are limited, specifically primary productivity is limited. Moving downstream, invertebrate richness increases as available habitat increases and diversifies. Many studies have documented invertebrate richness and community assemblage variation throughout stream and river systems and they
attribute richness and distribution to many variables; longitudinal distribution, habitat size, primary productivity, temperature, water velocity, substrate composition, and invertebrate predation (Barquin and Death 2011; StSaviour 2011). Although seasonal variation in aquatic invertebrate numbers has not been quantified in the Warm Springs area, StSaviour (2011) one summer survey in the Warm Springs area found invertebrate drift abundance to be negatively correlated with temperature, and positively correlated with water discharge. Invertebrate abundance was greater in cooler, downstream reaches of the Muddy River (StSaviour 2011), the historic habitat of Moapa dace. There may be an optimum temperature, or several environmental factors that interact to create optimum invertebrate diversity and richness in the Warm Springs Area. Quantifying invertebrate richness and abundance spatially and temporally could provide support for the hypothesis that decreases in invertebrate abundance is contributing to reduced dace survival in the Warm Springs area. Survival was positively associated with longer fork lengths up to fork lengths of 55 mm (Fig.5). Growth rates and thus fork length in fishes are variable among individuals and can be attributed to variation in prey availability, water temperature, flow, dissolved oxygen, genetics, and social interactions (Brett 1979, Wootton 1990, Benjamin et al. 2014). Despite the numerous factors that influence fork length, body size is also dependent upon age, especially in the first year (Brett 1979, Wootton 1990, Scoppettone and Burge 1994). Moapa dace confined to the upper 85 meters of Pederson tributary grew an average of 36 mm from age-0 to age-1 (Scoppettone and Burge 1994). Data addressing the relationship between age and fork length is limited because aging Moapa dace requires sacrifice and reliance on opercle bones because scales are very small, deeply imbedded, and fail to show annual circuli markings
Fork length seems to be a good indicator of Moapa dace age, though only eight individuals were used to describe this relationship (Scoppettone et al. 1992). The observed quadratic relationship of fork length and survival suggests that Moapa dace survival is low in the first year of life, highest at intermediate body sizes, and decreases later when senescence reduces survival. If adult dace are able to recolonize the Muddy River, it is likely that the shape of this trend will expand to incorporate larger body sizes that were common in the river. The first study to estimate annual survival of Moapa dace found survival of larvae (7 - 10 mm) to one year (41 - 54 mm; hereafter first year) to be 0.32 (n = 125) and survival of second year fish to be 0.60 (n = 43) in the upper 85 meters of Pederson tributary (Scoppettone and Burge 1994). In their study, Scoppettone and Burge transplanted 125 larval dace into Pederson Stream immediately after piscicide treatment so the stream was devoid of fishes prior to the transplant (1994). To derive survival estimates Scoppettone and Burge quantified survival as the number of individuals at time two divided by the number of individuals at time one. This study found that annual Moapa dace survival varied among populations, years, and by fork length, with Upper Pederson (the population that Scoppettone and Burge studied) having intermediate survivals in comparison to other populations (Fig. 7). While it is not possible to directly compare estimates from these two studies, the annual survival estimate for first year dace from Scoppettone and Burge (1994) is approximately equal to the annual survival ($\phi_a$) estimates of Moapa dace that are 40 mm ($\phi_a = 0.276$), generated in this study. Contemporary survival estimates suggest that bimonthly survival and annual survival is much lower for fish less than 40 mm, suggesting that actual estimates of larvae to one year is much lower than 0.276 (Fig.5). I captured few dace
smaller than 31 mm FL (n = 120) so the ability to estimate survival for these smallest fork lengths is limited (Fig. 5). It is likely that some of the variation between past and contemporary estimates can be explained by differences in study design and habitat conditions. Moapa dace larvae studied by Scoppettone and Burge (1994) were restricted to the upper reaches of Pederson stream, and were not exposed to any competition with larger conspecifics, native Moapa White River spring fish (Crenichthys baileyi), or non-native species such as short fin molly or mosquitofish. In contrast, Moapa dace in the study were cohabitating with conspecifics and interspecifics which increased competition for space and food resources, and likely reduced survival. Moapa dace larvae occur most often in the upper sections of spring outflows (Scoppettone et al. 1992), suggesting that high first year survival estimates generated by Scoppettone and Burge (1994) may have been a product of ideal habitat conditions and no competition with other larger fishes. Moapa dace recruitment varied seasonally and among populations over the duration of this study. Moapa dace recruited into the population when they became a trappable size, typically 30 - 50 mm fork length. Larval dace were not captured and not included in this analysis. Recruitment occurred year round at low levels (0.06 - 0.13) but was significantly higher in April and June (0.21 – 0.48). This finding was somewhat expected because other studies have documented eggs in skeins at various stages of development and observed Moapa dace larvae year round with larval abundance greatest in April and May (Scoppettone et al. 1992). What was surprising was the intensity of the recruitment peak, which can be attributed to larval fish from the previous year recruiting to a catchable size. In this study, all dace that reached a trappable size were considered to be part of the adult age class depicted in lambda estimates. Lambda was > 1 in Upper Apcar,
Lower Apcar, and Lower Pederson in 2011 while other populations were experiencing slight population declines. In 2012, the rate of population change was substantially greater in all populations (Fig. 9). These findings coincide with snorkel abundance estimates that observed similar trends within these populations including gradual increases in abundance in Upper Apcar and Lower Apcar in 2011 and gradual or substantial increases in abundance in all populations in 2012 (Simons, unpublished data). Heterozygosity at eight loci failed to explain more variation than the constant survival model. In contrast, heterozygosity at ten loci improved the fit of the model and this predictor was included in the best group and time survival model with other explanatory covariates. However, heterozygosity at ten loci had a modest effect on survival (Fig. 5). Other studies have concluded that heterozygosity and fitness are correlated because the neutral genetic variation found at microsatellite loci is thought to be indicative of allozyme variation (DeWoody and Avise 2000). However, in this study null alleles were detected at the two additional loci that were included in the calculations of heterozygosity at ten loci. Null alleles are detected when the primer sequence of a microsatellite region has mutated, resulting in amplification failure at the mutated loci (Hallerman 2003). Individuals that have one copy of the mutation are counted as homozygous, even if they are actually heterozygous because only one locus copy is effectively amplified by PCR. As a result, the effect of the null alleles on the estimation of heterozygosity is to produce a biased low estimate when the null alleles are present. Therefore, individuals that were actually homozygous at one or both of these loci were treated the same as individuals that had null alleles at one or both of these loci. Typically the presence of null alleles is thought to obfuscate detection of the heterozygosity-fitness correlate because no PCR
product is produced (David 1998, Hallerman 2003). Even though I do not know of any functional significance for these microsatellite loci, the mutation leading to a null allele could involve a relatively large deletion that could have functional significance. A large deletion could explain the observed reduction of survival and fitness in individuals with lower heterozygosities when null alleles are included in the overall heterozygosity calculation. In this case, it is impossible to assess whether the homozygotes are truly homozygotes or just a result of the null alleles. The individuals in this study would have to be genotyped at additional loci to rigorously test for a heterozygosity effect. While samples of tagged fish were relatively small, tags had a strong negative effect on survival ($\beta = -1.03 \pm 0.20$). Tagged dace had lower bimonthly survival than non-tagged dace by 0.06 - 0.13 in Upper Apcar and 0.10 - 0.19 in Upper Plummer over the six and ten month durations when tags were deployed (Fig. 6). Annually, tagged dace had lower survival than non-tagged dace by 0.30 in Upper Apcar and 0.33 - 0.38 in Upper Plummer.

Reduced survival among tagged individuals is especially surprising because I only tagged Moapa dace that were > 49 mm fork length, ≥ one gram, and healthy in appearance. Additionally, I found bimonthly survival to be highest (> 0.90) among dace 49 - 62 mm fork length and most (70%) of the dace I tagged were 50 to 62 mm fork length at the time of tagging. Only four individuals (5%) were greater than 70 mm, a group whose bimonthly survival was found to less than 0.80 based on their fork length. These tags were the smallest available transponder tags- still, for the smallest individuals; they were 14% of their body length. It is expected that the large size of these tags relative to dace length contributed to the reduced survival of Moapa dace. Other studies have found passive integrated transponder (PIT) tags to have differential effects on survival (Ficke et
al. 2012). Survival is usually reduced though the extent of reduction varies by tag size, fish size, and fish species (Ficke et al. 2012). Tagged dace were also anesthetized with tricaine methanesulfonate (MS222). Use of MS222 can result in fish mortalities (Carter et al. 2011), however I took all precautions to accurately mix MS222 and limit dace exposure to the solution. I also ensured all individuals recovered from anesthesia prior to release, however it is possible that the MS222 negatively impacted survival. Still, it is more likely that the tag itself was the main cause of reduced survival because other studies have found small reductions in survival from exposure to MS222 and false tagging, and much larger reductions in survival of fish that were exposed to MS222 and implemented with a tag (Ficke et al. 2012). To the best of my knowledge, no other studies have specifically tested these transponder tags so I suggest a controlled lab experiment using a less sensitive to adequately assess the costs and possible benefits associated with using transponder tags for such small fish. For the purposes of this study, genetic tagging using ten polymorphic microsatellite loci was an effective, efficient, and economical means of identifying individuals. The use of microsatellites to identify individuals is recommended for sensitive or endangered species.

**Conservation Implications**

Dramatic fluctuations in abundance are common for fish populations however; abundance fluctuations for endangered species can be a real threat to species persistence. By understanding how survival, recruitment, and environmental correlates contribute to population change, managers can better understand observed trends and adapt management to target life stages deemed most sensitive to population persistence and
growth. I found spatial and temporal variation in survival and recruitment. Survival also varied by fork length and increased over the three year study. These results can be attributed to the Moapa dace population rebounding from population lows observed in 2008 and 2009. Increases in survival over the three year study resulted in increases in population growth. Despite increases in lambda, recruitment did not increase among years. It is possible that habitat limitations are creating high levels of intraspecific competition among larvae, juvenile, and adult Moapa dace, which is limiting recruitment. If managers want to see increases in recruitment they should continue to restore habitat in Apcar, Pederson and Plummer, but more importantly, they should remove the downstream barrier and make the Muddy River available for dace. Additionally, restoring other tributaries such as the North Fork and South Fork will create more habitats specifically for recruitment. If Moapa dace remain restricted to their current range, they will likely reach the maximum carrying capacity of these tributaries and population growth will remain limited. However, if downstream habitat and other headwater tributaries of the Muddy River become available, Moapa dace population dynamics will likely realize that of historic populations where Moapa dace grew larger, were more fecund, and proliferated in the warm waters of the Muddy River and its tributaries. Assuming successful tilapia extirpation, expansion of available habitat will only benefit the Moapa dace. In addition to increasing habitat, restoring natural flow regimes and eliminating non-native species are effective ways to ensure the expansion and success of Moapa dace.
LITERATURE CITED


StSaviour, A. 2011. A food web analysis of a Mojave desert geothermal spring system and feeding ecology of Moapa dace (*Moapa coriacea*). (Masters thesis) Oregon State University.


TABLE LEGENDS

Table 1. Microsatellite loci used for on Moapa dace genetic analysis, their primer sequences, literature sources, range of allele sizes, expected (He) and observed (Ho) heterzygosities and allelic richness (R_s). Table 2. Summary of general, temporal, spatial, and individual covariate models that were tested for Moapa dace captured between October 2009 and September 2012.

Table 3. Performance of Pradel survival and recruitment models of Moapa dace detection (p), survival (ϕ), and recruitment (f) based on captures from minnow traps between October 2009 and September 2012 in the Apcar, Pederson, and Plummer tributaries in Clark County, NV. Considered models are ranked by quasi-Akaike's Information Criterion adjusted for small sample size (QAIC_c). Probability that each model is the best model is given in QAIC_c weight (wt). The number of parameters (K) and model deviance (QDeviance) are also presented.

Table 4. Models of lambda using Pradel recruitment and lambda models for Moapa dace captured in minnow traps between October 2009 and September 2012 in Apcar, Pederson, and Plummer tributaries of the Muddy River in Clark County, NV. Detection and survival models are p(g + t + TN + TAG + PG) phi(g.A + Y + sea + TAG). Considered models are ranked by quasi-Akaike's Information Criterion adjusted for small sample size (QAIC_c). Probability that each model is the best model is given in QAIC_c weight (wt). The number of parameters (K) and model deviance (QDeviance) are also presented.
FIGURE LEGENDS

Figure 1. Current hydrology of the Muddy River which consists of more than 20 geothermalsprings known as the Warm Springs area in Clark County, Nevada.

Figure 2. Detail map of study site and current range of Moapa dace. Apcar, Pederson, and Plummer tributaries are part of a larger geothermal spring network which combined, provides the headwaters of the Muddy River. Populations are differentiated by tributary, property boundaries, and physical barriers to Moapa dace. Populations are APL (red)-Upper Apcar, APL (purple)-Lower Apcar, PEU (dark blue)-Upper Pederson, PEC (light blue)-Lower Pederson, PLU (yellow)-Upper Plummer, and PLL (green)-Lower Plummer.

Figure 3. Bimonthly encounter probability and 95% confidence intervals from most parsimonious model of Moapa dace populations between October 2009 and September 2012 in the Warm Springs area, Clark County, NV. The most parsimonious encounter probability model included additive spatial and temporal variation among all six stream segment populations and all 18 sampling periods. The model accounted for the effect of the transponder tag and two sampling variance parameters, the number of traps set, and the percent of individuals genotyped.

Figure 4. Spatial and temporal variation in bimonthly survival and 95% confidence intervals from the most parsimonious model of Moapa dace captured between October 2009 and September 2012 in the Warm Springs area, Clark County, NV. This model depicts additive variation among five stream populations with year and seasonal trends. Population boundaries depicted in Fig. 2 include APU-Upper Apcar, APL & PEC-Lower Apcar and Lower Pederson, PEU-Upper Pederson, PLU-Upper Plummer, PLL-Lower Plummer.

Figure 5. Fork length squared (FL²) and heterozygosity at ten loci explained some of the variance in the most parsimonious bimonthly survival model as individual covariates. Moapa dace bimonthly survival as it varied with fork length- FL² (A) and heterozygosity at ten loci (B). Number of individuals of Moapa dace captured within different size classes (C) and their heterozygosities (D) at ten loci for Moapa dace captured between October 2009 and September 2012 in the Warm Springs area, Clark County, NV. Solid lines indicate mean survival; dashed lines indicate 95% confidence intervals.

Figure 6. Bimonthly survival of Moapa dace tagged with transponder tags in Upper Apcar (APU + TAG) (n = 16) and Upper Plummer (PLU + TAG) (n = 53) in
comparison to control (not tagged) Moapa dace in Upper Apcar and Upper Plummer between March 2011 and September 2012. These estimates derived from fixing the user specified covariates to exclude tagged dace, or to include only tagged dace.

**Figure 7.** Annual survival probability and 95% confidence intervals for non-PIT tagged Moapa dace in 2010 (November 2009 - October 2010), 2011 (November 2010 - October 2011), and 2012 (November 2011 - October 2012*) captured in the Warm Springs area, Clark County, NV. October 2012 survival estimate extrapolated from most parsimonious model.

**Figure 8.** Seasonal recruitment and 95% confidence intervals for Moapa dace between October 2009 and September 2012 in the Warm Springs area, Clark County, NV.

**Figure 9.** Annual rate of population growth of Moapa dace populations between October 2009 and September 2012 in the Warm Springs area, Clark County, NV. Estimates are based on non-NANO tagged individuals only.
<table>
<thead>
<tr>
<th>Locus</th>
<th>PCR</th>
<th>Core motif</th>
<th>PRIMER SEQUENCE (5’-3’)</th>
<th>Source</th>
<th>Genus species</th>
<th>Common name</th>
<th>Range</th>
<th>H_e</th>
<th>H_o</th>
<th>Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee-C1</td>
<td>PCR1</td>
<td>(CA)16</td>
<td>F: AGGTGTTGGTTCCTCCCG</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>142-182</td>
<td>0.86</td>
<td>0.86</td>
<td>12.57</td>
</tr>
<tr>
<td>AY962241</td>
<td>One</td>
<td>VIC</td>
<td>R: TGGTATCTCGGTTTCACGAC</td>
<td>Debut et al. 2009</td>
<td>Leuciscus leuciscus</td>
<td>Eurasian Dace</td>
<td>187-209</td>
<td>0.66</td>
<td>0.65</td>
<td>4.08</td>
</tr>
<tr>
<td>LleB-072</td>
<td>PCR1</td>
<td>(TG)13</td>
<td>F: TCATTAGGGAAGCTTGTTATTC</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>207-241</td>
<td>0.25</td>
<td>0.25</td>
<td>3.57</td>
</tr>
<tr>
<td>FJ601720</td>
<td>One</td>
<td>NED</td>
<td>R: CTTTTCAACATTTGTCACGG</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>255-297</td>
<td>0.90</td>
<td>0.89</td>
<td>16.07</td>
</tr>
<tr>
<td>Lsou08</td>
<td>PCR2</td>
<td>(GT)17</td>
<td>F: GCCTGGAACCGCTTAACCT</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>250-334</td>
<td>0.91</td>
<td>0.91</td>
<td>18.93</td>
</tr>
<tr>
<td>EF209003</td>
<td>One</td>
<td>FAM</td>
<td>R: TGGGAACGAAAGGCGCTTG</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>262-312</td>
<td>0.81</td>
<td>0.44</td>
<td>15.85</td>
</tr>
<tr>
<td>LleC-090</td>
<td>PCR2</td>
<td>(TC)3:GG(TC)3</td>
<td>F: TCAAGACAAACTAAACGACC</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>233-265</td>
<td>0.83</td>
<td>0.40</td>
<td>8.68</td>
</tr>
<tr>
<td>FJ601722</td>
<td>One</td>
<td>VIC</td>
<td>R: GGCCTGTCAGGACACTGA</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>256-282</td>
<td>0.61</td>
<td>0.62</td>
<td>9.80</td>
</tr>
<tr>
<td>Gbi-G34</td>
<td>PCR3</td>
<td>(GATA)14</td>
<td>F: GTCTCGGGGTCTCCAACCTC</td>
<td>Meredith and May 2002</td>
<td>Lahontan Tui Chub</td>
<td>Gila bicolor obesa</td>
<td>226-300</td>
<td>0.77</td>
<td>0.77</td>
<td>18.93</td>
</tr>
<tr>
<td>AF393666</td>
<td>One</td>
<td>FAM</td>
<td>R: GTGCTGCCCTGTCACCA</td>
<td>Meredith and May 2002</td>
<td>Lahontan Tui Chub</td>
<td>Gila bicolor obesa</td>
<td>226-312</td>
<td>0.81</td>
<td>0.44</td>
<td>15.85</td>
</tr>
<tr>
<td>Gbi-G39</td>
<td>PCR4</td>
<td>(GATA)11</td>
<td>F: GAGCCGGTGAGTTTTACTATTAT</td>
<td>Meredith and May 2002</td>
<td>Lahontan Tui Chub</td>
<td>Gila bicolor obesa</td>
<td>226-312</td>
<td>0.81</td>
<td>0.44</td>
<td>15.85</td>
</tr>
<tr>
<td>AF393668</td>
<td>Two</td>
<td>VIC</td>
<td>R: ATTCATTATCCGGGGTTCTCAT</td>
<td>Keeler-Foster et al. 2004</td>
<td>Bonytail Chub</td>
<td>Gila elegans</td>
<td>233-265</td>
<td>0.83</td>
<td>0.40</td>
<td>8.68</td>
</tr>
<tr>
<td>Gel_223</td>
<td>PCR4</td>
<td>(TATC)18</td>
<td>F: CATACGTGATTTTTAATTAAGCTTG</td>
<td>Keeler-Foster et al. 2004</td>
<td>Bonytail Chub</td>
<td>Gila elegans</td>
<td>233-265</td>
<td>0.83</td>
<td>0.40</td>
<td>8.68</td>
</tr>
<tr>
<td>AY2699262</td>
<td>Two</td>
<td>NED</td>
<td>R: GTTACTGTAAGTGTTGGAGAAC</td>
<td>Dowling et al. 2005</td>
<td>Roundtail Chub</td>
<td>Gila robusta</td>
<td>256-282</td>
<td>0.61</td>
<td>0.62</td>
<td>9.80</td>
</tr>
<tr>
<td>GilaD17</td>
<td>PCR4</td>
<td>(GT)13</td>
<td>F: TGGCAGGAAAAGAGAAACT</td>
<td>Dowling et al. 2005</td>
<td>Roundtail Chub</td>
<td>Gila robusta</td>
<td>256-282</td>
<td>0.61</td>
<td>0.62</td>
<td>9.80</td>
</tr>
<tr>
<td>N/A</td>
<td>Two</td>
<td>FAM</td>
<td>R: ATAAGAGAAGAGAAGAACT</td>
<td>Dowling et al. 2005</td>
<td>Roundtail Chub</td>
<td>Gila robusta</td>
<td>256-282</td>
<td>0.61</td>
<td>0.62</td>
<td>9.80</td>
</tr>
<tr>
<td>BL1-2b</td>
<td>PCR4</td>
<td>(TG)12</td>
<td>F: TTTGACACTAGTAACAGCAGCATA</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>172-202</td>
<td>0.54</td>
<td>0.56</td>
<td>4.84</td>
</tr>
<tr>
<td>FJ468347</td>
<td>Two</td>
<td>FAM</td>
<td>R: CAGCACAGTTTCACTCATCA</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>209-249</td>
<td>0.83</td>
<td>0.83</td>
<td>10.58</td>
</tr>
<tr>
<td>Lsou05</td>
<td>PCR5</td>
<td>(CA)17</td>
<td>F: CGCGAAGAGACCCTGTTGC</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>209-249</td>
<td>0.83</td>
<td>0.83</td>
<td>10.58</td>
</tr>
<tr>
<td>EF209002</td>
<td>Two</td>
<td>FAM</td>
<td>R: CCCACATGCTGACTCCTGAC</td>
<td>Debut et al. 2009</td>
<td>Eurasian Dace</td>
<td>Leuciscus leuciscus</td>
<td>209-249</td>
<td>0.83</td>
<td>0.83</td>
<td>10.58</td>
</tr>
</tbody>
</table>

*Florescent tag sequences on forward primers are VIC=gcggataacaatttcacacgg, NED=taaaacgacggccagtgc, FAM=tttccagctcagacgttg; Reverse tag=gtttctt
<table>
<thead>
<tr>
<th>Model Name</th>
<th>No. of Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group * Time</td>
<td>g * t</td>
<td>102 - 108</td>
</tr>
<tr>
<td>Group + Time</td>
<td>g + t</td>
<td>23 - 24</td>
</tr>
<tr>
<td>constant</td>
<td>.</td>
<td>1</td>
</tr>
</tbody>
</table>

**Temporal**

<table>
<thead>
<tr>
<th>Model Name</th>
<th>No. of Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>m</td>
<td>6</td>
</tr>
<tr>
<td>Season</td>
<td>sea</td>
<td>3</td>
</tr>
<tr>
<td>Season</td>
<td>sea2</td>
<td>3</td>
</tr>
<tr>
<td>Full time</td>
<td>t</td>
<td>17-18</td>
</tr>
</tbody>
</table>

**Spatial**

<table>
<thead>
<tr>
<th>Model Name</th>
<th>No. of Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>g</td>
<td>6</td>
</tr>
<tr>
<td>Appear Group</td>
<td>g.A</td>
<td>5</td>
</tr>
<tr>
<td>Pederson Group</td>
<td>g.P</td>
<td>5</td>
</tr>
<tr>
<td>System</td>
<td>sys</td>
<td>3</td>
</tr>
<tr>
<td>Property</td>
<td>pr</td>
<td>2</td>
</tr>
</tbody>
</table>

**Covariates**

<table>
<thead>
<tr>
<th>Model Name</th>
<th>No. of Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fork length</td>
<td>FL</td>
<td>1</td>
</tr>
<tr>
<td>Fork length²</td>
<td>FL²</td>
<td>2</td>
</tr>
<tr>
<td>Heterozygosity (8)</td>
<td>H8</td>
<td>1</td>
</tr>
<tr>
<td>Heterozygosity (10)</td>
<td>H10</td>
<td>1</td>
</tr>
<tr>
<td>Tag presence</td>
<td>TAG</td>
<td>1</td>
</tr>
</tbody>
</table>

**Sample Variance**

<table>
<thead>
<tr>
<th>Model Name</th>
<th>No. of Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trap effort</td>
<td>TN</td>
<td>1</td>
</tr>
<tr>
<td>Percent genotyped</td>
<td>PG</td>
<td>1</td>
</tr>
</tbody>
</table>

*a not a priori models*
Table 3.

<table>
<thead>
<tr>
<th>Model notation</th>
<th>Group models</th>
<th>Time models</th>
<th>Time varying covariates</th>
<th>Individual time varying covariates</th>
<th>Individual covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
<td>$g + t + TN + TAG + PG$</td>
<td>$g.A$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$0.00$</td>
<td>$0.99988$</td>
<td>$46$</td>
<td>$14805.76$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$18.84$</td>
<td>$0.00008$</td>
<td>$44$</td>
<td>$14828.73$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$20.62$</td>
<td>$0.00004$</td>
<td>$45$</td>
<td>$14864.21$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$72.99$</td>
<td>$0.00000$</td>
<td>$40$</td>
<td>$14891.11$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$198.55$</td>
<td>$0.00000$</td>
<td>$30$</td>
<td>$15037.15$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$214.04$</td>
<td>$0.00000$</td>
<td>$36$</td>
<td>$15040.36$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$226.28$</td>
<td>$0.00000$</td>
<td>$32$</td>
<td>$15060.80$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$276.52$</td>
<td>$0.00000$</td>
<td>$29$</td>
<td>$15117.16$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$314.48$</td>
<td>$0.00000$</td>
<td>$29$</td>
<td>$15155.12$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$318.69$</td>
<td>$0.00000$</td>
<td>$30$</td>
<td>$15157.29$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$320.35$</td>
<td>$0.00000$</td>
<td>$30$</td>
<td>$15158.95$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$322.86$</td>
<td>$0.00000$</td>
<td>$26$</td>
<td>$15226.41$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$330.98$</td>
<td>$0.00000$</td>
<td>$28$</td>
<td>$15173.66$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$332.99$</td>
<td>$0.00000$</td>
<td>$29$</td>
<td>$15173.63$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$379.66$</td>
<td>$0.00000$</td>
<td>$26$</td>
<td>$15226.41$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$398.64$</td>
<td>$0.00000$</td>
<td>$26$</td>
<td>$15245.39$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$422.98$</td>
<td>$0.00000$</td>
<td>$26$</td>
<td>$15269.74$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$444.90$</td>
<td>$0.00000$</td>
<td>$25$</td>
<td>$15293.68$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$599.87$</td>
<td>$0.00000$</td>
<td>$20$</td>
<td>$15458.81$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$680.30$</td>
<td>$0.00000$</td>
<td>$8$</td>
<td>$15563.48$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$691.68$</td>
<td>$0.00000$</td>
<td>$4$</td>
<td>$15582.89$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$723.90$</td>
<td>$0.00000$</td>
<td>$4$</td>
<td>$15615.11$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$730.49$</td>
<td>$0.00000$</td>
<td>$4$</td>
<td>$15621.70$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$761.66$</td>
<td>$0.00000$</td>
<td>$4$</td>
<td>$15652.87$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$823.08$</td>
<td>$0.00000$</td>
<td>$3$</td>
<td>$15716.30$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
<tr>
<td>$g + t + TN + TAG + PG$</td>
<td>$824.97$</td>
<td>$0.00000$</td>
<td>$4$</td>
<td>$15716.18$</td>
<td>$g.A + Y + sea + FL^2 + TAG + H10$</td>
</tr>
</tbody>
</table>

*Model notation Group models: $g$-six populations differentiated by different stream segments (Fig.2), $g.A$-five populations, PEC and APL combined, $g.P$- five populations, PEU and PEC combined. Time models: $t$-varies by sampling period, $m$- bimonthly, six per year, $sea$- seasonal trend, three per year; Oct&Dec, Feb&Apr, and Jun&Aug, $sea^2$- Dec&Feb, Apr&Jun, and Aug&Oct; $Y$-year. Time varying covariates: $TN$-trap count, varied by sampling period and reach, $TH$- sum of trapping hours, varied by sampling period and reach. $PG$- percent genotyped, varied by sampling period and reach. Individual time varying covariates: $FL$-standardized forklength, $FL^2$-std. FL-squared. $TAG$- transponder tag presence accounted for. Individual covariates: $H8$-heterozygosity at eight loci, $H10$- heterozygosity at ten loci. + indicates additive term, (.) indicates parameter was held constant.
<table>
<thead>
<tr>
<th>λ*</th>
<th>Δ QAICc</th>
<th>QAICc wt</th>
<th>K</th>
<th>QDeviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>g.A + Y + m</td>
<td>0.00</td>
<td>0.99969</td>
<td>44</td>
<td>14942.29</td>
</tr>
<tr>
<td>g.A + m</td>
<td>16.16</td>
<td>0.00031</td>
<td>42</td>
<td>14962.56</td>
</tr>
<tr>
<td>g.A</td>
<td>33.64</td>
<td>0</td>
<td>37</td>
<td>14990.31</td>
</tr>
<tr>
<td>g</td>
<td>35.56</td>
<td>0</td>
<td>38</td>
<td>14990.19</td>
</tr>
<tr>
<td>g.P</td>
<td>46.27</td>
<td>0</td>
<td>37</td>
<td>15002.95</td>
</tr>
<tr>
<td>sys</td>
<td>66.49</td>
<td>0</td>
<td>35</td>
<td>15027.26</td>
</tr>
<tr>
<td>pr</td>
<td>128.94</td>
<td>0</td>
<td>34</td>
<td>15091.76</td>
</tr>
<tr>
<td>m</td>
<td>151.66</td>
<td>0</td>
<td>38</td>
<td>15106.28</td>
</tr>
<tr>
<td>sea</td>
<td>155.00</td>
<td>0</td>
<td>35</td>
<td>15115.77</td>
</tr>
<tr>
<td>Y</td>
<td>159.87</td>
<td>0</td>
<td>35</td>
<td>15120.64</td>
</tr>
<tr>
<td>(.)</td>
<td>165.19</td>
<td>0</td>
<td>33</td>
<td>15130.06</td>
</tr>
</tbody>
</table>

*Model notation Group models: g-six populations differentiated by different stream segments (Fig.3), g.A-five populations, PEC and APL combined, g.P- five populations, PEU and PEC combined, sys- 3 populations, Apacar, Pederson and Plummer, pr- property boundaries, upper and lower. Time models: m- bimonthly, six per year, sea- seasonal trend, three per year; Oct&Dec, Feb&Apr, and Jun&Aug, Y-year. + indicates additive term, (.) indicates parameter was held constant.
Figure 2.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.
Figure 9.