7-1-2015

Conservation Genetics at the Interface of Theory and Application

Tyler J. Pilger

Tyler J. Pilger
Candidate

Department of Biology
Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Thomas F. Turner, Chairperson

Keith B. Gido

Vaishali Katju

David L. Propst

CONSERVATION GENETICS AT THE INTERFACE OF THEORY AND APPLICATION

by

TYLER JESS PILGER

B.S., Bioloy, Fort Hays State University, 2006
M.S., Biology, Kansas State University, 2009

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy

Biology

The University of New Mexico
Albuquerque, New Mexico

July, 2015

ACKNOWLEDGMENTS

Many funding sources made this dissertation research possible, including support from an undergraduate fellowship from the National Science Foundation through the Undergraduate Opportunities Program (NSF-DEB 0731350), a New Mexico Department of Game and Fish (NMDGF) Share-with-Wildlife grant, a state wildlife grant administered by NMDGF and United States Fish and Wildlife (USFWS), and a WaterSMART grant from the Desert Landscape Conservation Cooperative administered by the United State Bureau of Reclamation (BOR). A number of federal, state, and non-governmental organizations helped make this research possible by providing site access to the Gila National Forest and Wilderness Areas (US Forest Service), the Gila River Farm conservation area (The Nature Conservancy), the Heart Bar Wildlife Management Area (NMDGF), and access to Blue Creek and Sunset Canal Diversion was provided by BOR and private ranchers.

I would also like to acknowledge the facilities and support provided by the University of New Mexico, Department of Biology. Tissues were collected under NMDGF permit 3015 (TFT) and stored at the Museum of Southwestern Biology Division of Fishes until processing for which Lex Snyder was an invaluable asset. Research followed Institutional Animal Care and Use Committee (IACUC) protocol 10-100492-MCC. Invaluable technical support was provided by George Rosenberg from the University of New Mexico Molecular Biology Facility, which is supported by NIH grant P20GM103452.

I sincerely thank my major advisor, Thomas Turner whose mentorship has been crucial for honing my scientific and investigative skills. I also greatly appreciate the time and effort provided by my committee members Vaishali Katju, Bruce Milne, David Propst and Keith Gido. James Whitney was instrumental in carrying out field work and providing the ecological foundation for this dissertation.

I also must thank current and previous members of the Turner lab for providing an atmosphere of camaraderie and inquisitive discussion, including, Megan Osborne, Evan Carson, Mike Schwemm, Nate Franssen, Rosalee Reece, Adam Barkalow, Tom Kennedy, Tracy Diver, Trevor Krabbenhoft, Corey Krabbenhoft, Jesse Trujillo, and Steve Ross. My experience at UNM has been enriched by you all.

Lastly, I wish to thank my parents for their continued support and constant encouragement.

CONSERVATION GENETICS AT THE INTERFACE OF THEORY AND APPLICATION

by

Tyler J. Pilger

B.S., Biology, Fort Hays State University, 2006
M.S., Biology, Kansas State University, 2009
Ph.D. Biology, University of New Mexico, 2015

ABSTRACT

Understanding the ecological mechanisms responsible for patterns of spatial genetic structure and diversity is a central issue to evolutionary ecology and biodiversity conservation. The Anthropocene has seen a mass extinction only previously observed through geological records, and freshwater fishes of North America have not been spared owing to large-scale modification of freshwater habitats and introduction of nonnative species. Concomitant with reduced numbers of species is a rapid reduction in genetic diversity within species; this diversity that is required for species to adapt to rapidly changing environments of human dominated landscapes. However, understanding why species exhibit different patterns of spatial genetic structure and genetic diversity requires substantial ecological data and knowledge of species' life histories. This body of research incorporates both ecological and genetic data to address key issues related to the conservation of native fishes of the upper Gila River, NM, USA, and evaluates how differences in ecology among species influences their evolutionary trajectories (i.e., genetic diversity and structuring).

Chapter 1 adopted a conservation genetics approach to evaluate the genetic health and long-term maintenance of genetic diversity of three imperiled species protected by New Mexico State and United States Federal laws. Estimates of contemporary effective size were low for these species, as were estimates of genetic structure (all species F_{ST} < 0.025) suggesting moderate to high gene flow for all species. Chapter 2 broadened the scope of focal species by including most of the fish community and increasing life history variation to evaluate how dispersal and life history influence patterns of genetic structure within a shared riverscape (i.e., attributes of a landscape specifically related to networks of streams and rivers). A key result was that genetic patterns were highly variable among species and related to life history and abundance. Across species, overall genetic differentiation (F_{ST}) was not strongly predicted by species traits, but fecundity was negatively associated with effect of distance on genetic structure (measured by Mantel's *r*). Chapter 3 examined the relationship between metapopulation processes and species evolutionary trajectories. Metapopulation genetic effective size was reduced by temporal instability (extirpation/recolonization), but high abundance appeared to counter balance effects of temporal instability. These results indicate that ecological trade-offs related to life-history strategies (e.g., fecundity, body size, parental investment, etc.) also influence individual species' evolutionary responses (i.e., genetic diversity and differentiation) to landscape factors and threats to persistence.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

The biodiversity crisis of the 21st century has been described by many without hyperbole, as the sixth major extinction event of Earth's history. Human domination of Earth's ecosystems (e.g., deforestation and landscape conversion to agriculture, damming of rivers, global translocations of species, and overexploitation) have left many species facing two options, adapt to rapidly changing environments or go extinct. Freshwater fishes of North America are no exception with nearly 61 contemporary fish taxa presumed extinct and approximately 700 additional taxa reported as vulnerable to extinction (Jelks et al. 2008). Furthermore, extinction rates of freshwater fish are expected to exceed those of terrestrial taxa (Ricciardi & Rasmussen 1999). Yet, species richness is only one facet of biodiversity being lost as freshwater ecosystems are transformed at an alarming rate. Coinciding with decreased population sizes and increased isolation of populations on human engineered landscapes, is a rapid loss of genetic variation, that which itself, is necessary for species to adapt to novel environments (Frankham 2005; Frankham et al. 2009). A major challenge facing species conservation is a general lack of incorporation of species' genetic health in conservation plans (Moyle et al. 2003). Specifically, how factors that impact species abundances and population connectivity influence species' evolutionary trajectories, should be a common question discussed among researchers and resource managers. But in asking this question, researchers must identify how differences in species ecologies are related to different patterns of genetic connectivity and their influence on genetic diversity. My dissertation research, which has been part of a collaborative research effort among myself, K. Gido, D. Propst, T. Turner and J. Whitney, has made great strides towards

filling this informational gap on how differences in ecologies influence species' evolutionary responses to threats facing many imperiled freshwater fish.

The genetic effective size (N_e) is an important metric for estimating a species' evolutionary trajectory because it provides an indication of the relative strengths of microevolutionary processes acting on a species (Frankham et al. 2009). N_e for a natural population is the number of interbreeding individuals that experience the same rate of genetic drift as that of a Wright-Fisher idealized population of size N_e. The field of Conservation Genetics has provided valuable information regarding how species abundances are related to N_e (e.g., Osborne et al. 2010) and has provided guidelines for the amount of genetic variation, in terms of N_e, necessary to maintain long-term genetic viability (e.g., Frankham et al. 2014). Whereas N_e is a conceptually simple metric, its estimation in natural populations is complex and there remains mixed empirical evidence that ecological differences among species can influence N_e. For an example of the complexity related to estimating N_e, consider a hypothetical population of fish in a large pond that live for one year and randomly mate to produce the next generation. Estimating N_e of this population is quite straightforward; by estimating the number of breeding individuals. Equally important and straightforward, is knowing what affects N_e; the number of breeding individuals. However, this important metric becomes more difficult to estimate and make predictions about when this same population of fish is divided into four smaller ponds of equal size interconnected by streams which can allow movement of individuals, but themselves cannot support populations. In such a case, population genetic theory says that N_e of all populations relies on a balance between the N_e of each pond and the rate of gene flow among ponds. If all ponds have equal N_e and equal gene

flow among them, then theory predicts that the overall N_e will be greater than the sum of the N_e of all ponds (Wright 1943). The act of subdivision, per se, reduces the probability of a given allele going to global fixation, thereby increasing N_e of the species. Unfortunately for managers and researchers alike, the species we are charged with protecting do not behave in such an idealized manner. Species' N_e is generally reduced by overlapping generations, asymmetric genetic connectivity, and extirpations, thereby further complicating our understanding of how to estimate and predict what will influence N_e. Yet, again theory dictates that N_e must be a function of factors related to local N_e and factors related to patterns of gene flow across populations. The importance of these theoretical predictions to conservation of species cannot be overstated because levels of genetic diversity of a species are not only held within single interbreeding populations, but also across all populations; that is, at the metapopulation level. This requires management actions to distinguish between processes affecting genetic diversity at the local scale from those affecting genetic diversity at the metapopulation scale.

A large number of empirical and theoretical studies provide a firm understanding of how extrinsic factors, such as large scale geographic features and landscape properties, should affect patterns of genetic variation among species. These studies primarily conclude that environmental barriers to dispersal and gene flow increase isolation of populations and this effect can be observed over a variety of taxonomic groups (Avise 1992; Avise 2000). Moreover, species can respond differently to the same environmental barriers (Manel et al. 2003). Yet far fewer studies have focused on the species-specific traits related to differential responses of species. This key question of how species

respond differently to the same external environment has been the focus of my dissertation research.

In Chapter 1, I used a conservation genetics approach to lay out key issues regarding the genetic health and long-term persistence of genetic variation for three State and Federally listed imperiled fishes in the upper Gila River. A principal finding was all species exhibited low N_e, especially headwater chub (*Gila nigra*) a species afforded little federal protection. Another important finding was that altering genetic connectivity among spikedace (*Meda fulgida*) and loach minnow (*Tiaroga cobitis*) populations could have negative consequences for the long-term persistence of these species.

In Chapter 2, I broadened the scope of focal species to include the most abundant members of the Gila River fish community to further evaluate the relationship between intrinsic differences among species and differential patterns of genetic connectivity. Here I used genetic data from each species to characterize patterns of genetic connectivity and dispersal related life-history traits to test whether different intrinsic traits could lead to species-specific genetic responses on a shared landscape. Aspects of the riverscape (distance between populations and number of confluences) were expected to elicit shared responses across species but species-specific responses should be related to life-history traits influencing dispersal. Species exhibited a wide variety of spatial structuring, including near panmixia, isolation-by-distance, regional structuring, and patterns related to differences in local genetic effective size (N_e). Whereas the degree of genetic differentiation (F_{ST}) was not strongly predicted by species traits, how genetic variation was distributed on the landscape via distance (Mantel's *r*) was strongly associated with variation of fecundity across species. We observed that distance, as a mechanism for

isolation, has a stronger effect on low fecundity species than high fecundity species. These differential responses to the landscape may have different consequences for maintaining species evolutionary potential.

In Chapter 3, I delved into theoretical principles of metapopulation effective size to evaluate their predictions using species with very different metapopulation dynamics. Theory predicts metapopulation processes, such as extirpations and recolonizations, generally lead to decreased metapopulation genetic effective size, yet this prediction has rarely been tested empirically. I showed that both species abundances and probability of extirpation are linked to differences in metapopulation effective size by species-specific patterns of genetic connectivity identified in Chapter 2. Furthermore, both theory and empirical data provide support for the conclusion that ecological trade-offs related to different life-history strategies may have different consequences for maintaining metapopulation genetic effective sizes among species.

This body of research provides much needed empirical support for the theoretical predictions that extirpation/recolonization dynamics decrease metapopulation effective size but the magnitude of the effect depends on ecological characteristics related to dispersal (Whitlock & McCauley 1990; Whitlock & Barton 1997). These results also have direct conservation implications for maintaining genetic diversity of species with different life histories. For species exhibiting high temporal stability, the primary factor responsible for reducing N_e of the metapopulation is variance in N_e among local populations. Minimizing among population variance will slow the decay of genetic diversity. Alternatively, metapopulation N_e of a species exhibiting population turnover is affected by the number of colonizers. Therefore, maintaining dispersal corridors to allow

for maximum number of colonists will minimize decay of genetic diversity. Lastly, this research highlights the importance of incorporating both ecological and genetic data to offer greater insights into population genetic theory and provide valuable information concerning the maintenance of intraspecific genetic variation.

Literature Cited

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, 63, 62-76.

Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University Press.

Frankham R (2005) Genetics and extinction. Biological Conservation, 126, 131-140.

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to Conservation Genetics, Second edn. Cambridge University Press, New York.

Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation, 170, 56-63.

Jelks HL, Walsh SJ, Burkhead NM, Contreras-Balderas S, Diaz-Pardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS, Platania SP, Porter BA, Renaud CB, Schmitter-Soto JJ, Taylor EB, Warren ML (2008) Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries, 33, 372-407.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution, 18, 189-197.

Moyle LC, Stinchcombe JR, Hudgens BR, Morris WF (2003) Conservation genetics in the recovery of endangered animal species: a review of US endangered species recovery plans (1977–1998). Animal Biodiversity and Conservation, 26, 85-95.

Osborne MJ, Davenport SR, Hoagstrom CW, Turner TF (2010) Genetic effective size, N_e, tracks density in a small freshwater cyprinid, Pecos bluntnose shiner (*Notropis simus pecosensis*). Molecular Ecology, 19, 2832-2844.

Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. Conservation Biology, 13, 1220-1222.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. Genetics, 146, 427-441.

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, 44, 1717-1724.

Wright S (1943) Isolation by distance. Genetics, 28, 114-138.

Chapter 1

COMPARATIVE CONSERVATION GENETICS OF PROTECTED ENDEMIC FISHES IN AN ARID-LAND RIVERSCAPE

A version of this chapter was previously published in Conservation Genetics, *the final publication is available at Springer via http://dx.doi.org/ 10.1007/s10592-015-0707-3*

Abstract

Conservation genetic studies are challenged by the fact that populations of many imperiled species have experienced declines and fragmentation to the degree they no longer exhibit natural, self-sustaining metapopulation processes; characteristics of great importance to managers charged with their protection. Genetic patterns of species from minimally impacted systems can inform management practices for populations in more modified and fragmented systems. We assessed spatial and temporal patterns of intraspecific genetic diversity and differentiation using microsatellites for three imperiled fishes of the unfragmented upper Gila River, New Mexico, USA. Estimates of contemporary effective size were low for these species, but we observed little genetic evidence of inbreeding. Overall genetic structure was low (all species $F_{ST} < 0.025$) suggesting moderate to high gene flow for all species, but each exhibited different patterns of spatial structuring. *Gila nigra* (a candidate for listing under the Endangered Species Act) appears most at risk of short-term loss of genetic variation and local extinction relative to *Meda fulgida* or *Rhinichthys* (*Tiaroga*) *cobitis* (both federally

endangered) because *G. nigra* exhibited the lowest diversity, smallest effective size (N_e ~ 100) and temporally unstable population structure. *Meda fulgida* and *T. cobitis* exhibited temporally stable spatial structure related to riverscape features but connectivity among occupied habitats is threatened by a proposed diversion structure. Data from this comparatively pristine system can inform management of these species in fragmented portions of their ranges.

Introduction

Biota of stream ecosystems are among the most endangered worldwide (Strayer and Dudgeon 2010; Vorosmarty et al. 2010); the most pervasive threats to freshwater species are habitat alteration, invasive species, and water extraction (Allan and Flecker 1993; Richter et al. 1997). Fishes of southwestern North America have experienced elevated extinction risk due to demographic and evolutionary consequences of increasing rarity and extent of fragmentation (Fagan et al. 2002). Anthropogenic disturbance in the form of impoundments, road crossings, and irrigation diversions have led to fragmentation that reduces persistence of obligate aquatic organisms, especially those that exist as metapopulations (Fagan 2002). Additionally, modified habitats such as reservoirs and engineered stream reaches can often support predators at high densities, which can indirectly inhibit dispersal of fishes (Harvey et al. 2004). Direct and indirect disruption of natural dispersal can isolate populations by reducing gene flow and decreasing genetic diversity (Slatkin 1985; Wofford et al. 2005). As populations become isolated and smaller they become more susceptible to demographic and stochastic effects that tend to reduce genetic diversity, increase inbreeding, and eventually lead to local extirpation (Frankham 2005).

Successful conservation and management of species not only requires information about abundances and ecology, but also dispersal, local extirpation, and colonization (i.e., metapopulation processes), and how these affect levels of genetic diversity and differentiation. In lotic systems, metapopulation processes are constrained by riverscape architecture, for which, some classic metapopulation and gene flow models do not apply (Fagan 2002). As a consequence, conceptual models have been proposed for predicting how riverscape architecture should influence genetic connectivity of aquatic taxa with differing life history traits and dispersal capabilities (Hughes et al. 2009). The Stream-Hierarchy-Model (Meffe and Vrijenhoek 1988; Hughes et al. 2009) posits that genetic structuring of species that occur throughout a stream network reflects the dendritic nature of the network. For species that are limited to headwaters, population differentiation within a sub-catchment will depend on whether or not streams confluence within headwater habitats as proposed by the Headwater-Model (Finn et al. 2007; Hughes et al. 2009). A complicating factor for many imperiled species is that populations have already experienced depressed abundances and are fragmented to such a degree they no longer exhibit natural metapopulation processes. Therefore, characterizing genetic patterns from pristine or minimally-impacted systems is critical to help managers understand natural levels of spatial genetic structuring, gene flow, and other population features. Such knowledge can guide restoration of metapopulation dynamics and repatriation efforts into formerly occupied, and presumably restored, habitats (Lewis et al. 1996; Huxel and Hastings 1999).

Water demands for human activities in the southwestern United States have resulted in the Colorado River basin being one of the most engineered drainage basins in

the world (Fradkin 1981; Carlson and Muth 1989). One exception is the upper Gila River catchment in southwestern New Mexico, USA, which has no major impoundments and accordingly is a stronghold for a largely intact native fish fauna composed mainly of endemic species, including headwater chub *Gila nigra*, spikedace *Meda fulgida*, and loach minnow *Tiaroga cobitis*. Despite limited direct human modification of the physical landscape, these native fishes in the upper Gila River catchment have declined in abundance and distribution (Propst et al. 2008). Ongoing threats include nonnative species (specifically, yellow bullhead *Ameiurus natalis*, flathead catfish *Pylodictis olivaris*, and smallmouth bass *Micropterus dolomieu*) that prey on native species (Pilger et al. 2010). Additional threats include prolonged drought and increased wildfire risk due to climate change (Westerling et al. 2006; Seager et al. 2007, Whitney et al. in press); threats that may be exacerbated by a proposed diversion structure under the authority of the Arizona Water Settlement Act (2004).

Genetic information can provide important insights for long-term persistence probabilities and evolutionary consequences of habitat alteration, species invasions, and stochastic environmental events. Thus, our primary objective was to quantify standing levels of genetic diversity, contemporary genetic effective size (N_e), and fine-scale population structure of *G. nigra*, *M. fulgida*, and *T. cobitis* in the upper Gila River catchment, a comparatively unaltered system. In addition, we used genetic data collected from two consecutive years to evaluate temporal changes in genetic patterns. These data are important for initiating baseline genetic monitoring and to establish ecological and evolutionary criteria for restoration and repatriation. Under an adaptive management

framework, this baseline is critical to evaluate the efficacy of current and proposed management actions.

Materials and Methods

Study species

Gila nigra is part of a phylogenetically unresolved species complex (*G. intermedia*, *G. nigra*, and *G. robusta*) and is restricted to headwater streams of the Gila River drainage of Arizona and New Mexico (Minckley and DeMarais 2000) (Figure 1.1). However, its historical distribution in the Gila River of New Mexico remains unclear because of taxonomic confusion in historical records (New Mexico Department of Game and Fish 2006). Contemporary surveys (1980 to present) have documented *G. nigra* in headwater reaches of the Gila River drainage, but viability of these populations remains uncertain (Paroz et al. 2006). Significant genetic variation in mitochondrial haplotypes and nuclear genes has been observed among catchments suggesting historical isolation (Schwemm 2006), yet little is known of fine scale genetic structuring within populations. Currently, *G. nigra* is a candidate for listing under the Endangered Species Act (U.S. Fish and Wildlife Service 2006) and listed as endangered by the State of New Mexico (New Mexico Department of Game and Fish 2006).

Meda fulgida and *T. cobitis* are endemic to the Gila River Basin and were once common throughout the Gila River upstream of its confluence with Aqua Fria River, including the Verde River, Salt River, and San Pedro River catchments of southeastern and central Arizona and southwestern New Mexico (Figure 1.1). Both species have been eliminated from at least 90% and 80% of their historical ranges, respectively (Propst

1999 and references therein). Range wide variation in mitochondrial DNA and allozymes revealed strong divergence among river catchments occupied by *M. fulgida* and *T. cobitis* suggesting little gene flow among extant populations in different catchments (Tibbets and Dowling 1996). As with *G. nigra*, fine scale genetic structure and diversity of these species in the upper Gila River basin has yet to be evaluated. Both species have decreased in headwater reaches of the Gila River over the last decade (Propst et al. 2008) prompting their reclassification in 2012 as endangered rather than threatened (U.S. Fish and Wildlife Service 2012).

Sampling

The upper Gila River catchment of New Mexico has no major impoundments and a natural flow regime from its headwaters in the Black and Mogollon Mountain Ranges to the New Mexico/Arizona border (Propst et al. 2008). Upstream tributaries, including West, Middle, and East Forks of the Gila River are within the Gila National Wilderness Area (Figure 1.2) and are more pristine compared to other southwestern streams. We selected sample locations to include the extent of each species current distribution in the drainage thus representing > 160 km of the Gila River with an elevation range of nearly 900 m (1161 to 2059 m above sea level). We sampled for target species during June and July 2009 and again in October and November 2010 to evaluate spatial and temporal patterns of population genetic diversity and structure. During each sampling event, individuals of similar size, typically juveniles, for each species were collected to include only individuals of the same cohort. Individuals were collected at each site using a combination of electrofishing (Smith-Root Model 12 backpack shocker) and seining (4.6 x 1.2m, 3.2mm mesh). Tissue samples for DNA extraction were collected by clipping a

small portion (< 5 mm^2) of the caudal fin and preserving it in 95% ethanol. Sampled individuals were allowed to recover in buckets of fresh water and released at the capture site according to an approved institutional animal care and use protocol (UNM IACUC #: 10-100492-MCC).

Molecular methods

Genomic DNA was extracted from air-dried fin clips using standard proteinase-K digestion and standard phenol/chloroform extraction (Hillis et al. 1996). Microsatellite loci for each focal species (or very close relatives thereof) were available from previously published studies (Table A.1). Multiplex polymerase chain reactions (PCR) containing primers for up to three loci were optimized depending on annealing temperature, size range, and fluorescent label for rapid genotyping of individuals. PCR conditions, size fragment analysis, and scoring are described in Trujillo et al. (2012). Approximately 10% of samples from each species were reanalyzed and rescored for quality assurance purposes.

Intra-specific genetic diversity and effective size

We used standard population genetic summary statistics to quantify standing levels of genetic diversity and differentiation across the riverscape for each species (Frankham et al. 2009). Conformation to Hardy-Weinberg equilibrium (HWE) was tested with modified exact tests and G-tests for each locus pair combination within samples and a global test for linkage disequilibrium using GENEPOP (Raymond and Rousset 1995; Rousset 2008). We screened each locus for large allele dropout, null alleles, and scoring errors that could result from stuttering using MICRO-CHECKER

(Van Oosterhout et al. 2004). Microsatellite allele frequencies and diversity statistics including Nei's unbiased gene diversity (H_E; Nei 1987), observed heterozygosity (H_O), rarified allelic richness (A_R), and inbreeding coefficients (F_{IS}) were obtained using the computer program FSTAT (Goudet 1995). Allelic richness was estimated for sites where the number of individuals was greater than or equal to ten.

We estimated genetic effective population size (N_e) to assess the relative effects of genetic drift (a dominant evolutionary force in small populations) for each species using the linkage disequilibrium method (Hill 1981) implemented in LDNE (Waples and Do 2008) and the sibship method implemented in COLONY (Wang 2009) denoted with subscripts D and S, respectively. For each species, individuals were pooled across sample locations to estimate N_e. Allele frequencies that approach one or zero can bias N_{eD} (Waples 2006); therefore, LDNE calculates estimates after excluding all alleles with frequencies of less than an a priori specified critical value. We set the critical value to 0.02, such that alleles that were less than 2% were excluded (Waples and Do 2008). Upper and lower 95% confidence intervals for N_{eD} were calculated using a jackknife approach implemented in the program. COLONY uses maximum likelihood to estimate probabilities of full and half siblings of a sample of individuals taken from a population, from which N_{eS} can be estimated. A major assumption of the method is that individuals are sampled randomly from a single cohort in a population. We tested if our samples met this assumption by calculating mean relatedness (Queller and Goodnight 1989) among individuals within each sample location using GenAlEx (Peakall and Smouse 2012). We expected relatedness to be low (< 0.25) within a sample if the individuals were randomly

sampled but high if our sample came from only a few highly related individuals and would downwardly bias N_{eS}

These two measures of N_e are different but provide complementary insight into contemporary evolutionary processes affecting focal populations. N_{eD} provides an estimate that is based on correlations among allele frequencies, and as such, may be sensitive to genetic structure among samples (Waples and Do 2008, 2010). Conversely, N_{eS} estimates the number of parents that gave rise to the sampled offspring and is not sensitive to differences in allele frequencies among samples but is sensitive to non-random sampling (Wang 2009; Waples and Waples 2011).

Intra-specific population genetic structure

We quantified genetic structure for each species using Weir and Cockerham (1984) F-statistics. Global F_{ST} values and bootstrapped 95% confidence intervals were estimated using FSTAT to provide overall levels of genetic differentiation within species. Differentiation between sample sites was quantified with pairwise F_{ST} values estimated in Arlequin (Excoffier et al. 2005). Arlequin implements a permutation procedure to test the significance of all pairwise F_{ST} values (i.e., $F_{ST} > 0$). We tested each species for isolation-by-distance (IBD) using Mantel tests to evaluate the relationship of stream distance (in km) and linearized F_{ST} (Slatkin 1995; Rousset 1997). Stream distances among sample sites were estimated using Google Earth. Mantel tests were performed using R version 2.15.0 (R Core Team 2012). Species displaying an IBD pattern would indicate conditions of migration-drift equilibrium (Hutchison and Templeton 1999);

however, absence of this pattern does not imply non-equilibrium conditions (e.g., Hughes et al. 2009).

We also used a Bayesian approach to assess genetic structure using STRUCTURE (Pritchard et al. 2000). STRUCTURE analysis for each species included an admixture model with correlated allelic frequencies and sample locations as prior probabilities (Hubisz et al. 2009). Five independent runs with 50,000 burn-in iterations followed by 100,000 iterations were performed for each value of K (1 to total number of sites a species was collected from), where K represents the potential number of distinct genetic units. The most likely K value for each species was evaluated using the Evanno method (Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl and von Holdt 2012).

Temporal patterns of genetic diversity and structure

Patterns of genetic diversity, effective size, and differentiation were compared between 2009 and 2010 samples to assess temporal stability in genetic patterns that can indicate stability in local abundance across years and equilibrium between migration and genetic drift. Temporal stability of genetic patterns can also indicate robustness and/or uniformity of conclusions compared to inferences based on a snapshot in time (Waples 1998). However, spatial patterns of genetic structure can be disrupted by disturbance and other ecological factors that result in temporal instability (e.g., Apodaca et al. 2013).

Results

In 2009, individuals of all target species were collected from five locations (n = 237 individuals) and from eight locations in 2010 (n = 362 individuals), but no location

was occupied by more than two target species (Table 1.1). *Gila nigra* (n=149) were collected at five sites in upstream reaches (EF, WF1, WF2, MF, GM1) but only four had a sufficient number of specimens for genetic analyses (Table 1.1). Both *M. fulgida* (n = 265, 6 sites) and *T. cobitis* (n = 185, 4 sites) occurred at headwater locations (WF1, WF2, MF) and Gila River mainstem sites (GM1 to GM4) (Table 1.1).

All ten microsatellite loci were polymorphic in each species with number of alleles ranging from 4 to 42 for *G. nigra*, 10 to 30 for *M. fulgida*, and 3 to 62 for *T. cobitis* (Table A.1). *Gila nigra* exhibited no deviations from HWE after sequential Bonferroni correction (Holm 1979; Rice 1989). Locus (*Rhca15*) for *G. nigra* had an excess of homozygotes at one site in 2009, which could be caused by presence of null alleles. Nine of 90 locus-by-site comparisons for *M. fulgida* deviated from HWE after correction and were caused by an excess of homozygotes at four loci exhibiting the highest levels of polymorphism (*ParB5T*: 29 alleles, *ParB56MB*: 23 alleles, *ParB64ML*: 24 alleles, *Nme93*: 30 alleles). *Tiaroga cobitis* also exhibited deviations from HWE (10 of 70 locus-by-site comparisons) resulting from an excess of homozygotes at two highly polymorphic loci (*Rhca15*: 62 alleles and *Rhca24*: 25 alleles). Analysis with MICRO-CHECKER indicated the possible presence of null alleles that could be responsible for the excess homozygotes. Eight pairs of *G. nigra* loci were significant for non-independence (i.e., exhibited evidence of linkage disequilibrium) of which six were only significant at one location in one year and two pairs were significant at two locations in the same year. Ten pairs of loci for *M. fulgida* had significant tests for non-independence of which only one pair was significant at two locations but in different years. *Tiaroga cobitis* had two pairs of loci with significant tests at the same location in 2009. All loci

were retained for analyses because violations of assumptions were inconsistently distributed among loci, populations and years.

Intra-specific genetic diversity and effective size

Mean observed heterozygosity (H_O) for *G. nigra* across sites and years was 0.63 (range = 0.60 to 0.66), mean gene diversity (H_E) was 0.66 (0.62 – 0.69), and mean allelic richness (A_R) was 6.5 (5.8 – 6.7; Table 1.1) and no spatial variation in diversity statistics was observed. *Gila nigra* exhibited overall low F_{IS} (0.001 – 0.092 across sites) and low relatedness within samples (-0.049 – 0.061; Table 1.1). Mean H_O for *M. fulgida* was 0.76 (0.66 – 0.81), H_E was 0.82 (0.75 – 0.85), and A_R was 9.6 (7.8 – 11.2) across sites and years. We observed spatial variation in H_E and A_R across sites occupied by *M. fulgida*. Mean H_E among downstream sites was 9% greater than among upstream sites (H_E = 0.85 and 0.78, respectively), and A_R was 32% greater among downstream sites than upstream sites (A_R = 10.8 and 8.2, respectively). *Meda fulgida* exhibited low F_{IS} (0.013 – 0.132) and low relatedness within samples (-0.033 – 0.065). Mean H_O for *T. cobitis* was 0.69 (0.64 – 0.73), H_E was 0.73 (0.71 – 0.78), and A_R was 10.4 (10.0 – 11.0) across sites and years. No spatial variation in diversity was observed for *T. cobitis* as A_R was only slightly higher among downstream sites (mean A_R = 10.6) than the upstream site (mean A_R = 10.1) and H_E was slightly lower among downstream sites compared to upstream sites (H_E = 0.73 and 0.75, respectively). *Tiaroga cobitis* exhibited low F_{IS} (0.017 – 0.115) and low relatedness within samples (-0.049 – 0.029).

Gila nigra had the lowest estimates of N_e of the three species, with estimates from both analyses producing values ≤ 105 (Table 1.2). Both *M. fulgida* and *T. cobitis*

exhibited $N_e \geq 100$ with N_{eD} for *T. cobitis* in 2010 having the largest ($N_{eD} = 602$). For all species, estimates were dependent on analysis method because N_{eD} was consistently greater than N_{eS} (Table 1.2).

Intra-specific population genetic structure

Target species were present at more sites in 2010 than 2009; therefore we report here on spatial population structuring based on 2010 data and reserve the 2009 results for comparing temporal patterns (see below). All three species had significant, but low global F_{ST} values (all $F_{ST} < 0.025$; Table 1.3). *Gila nigra* and *M. fulgida* had similar levels of differentiation followed by *T. cobitis* exhibiting the least differentiation. Each species exhibited a different pattern of fine-scale structuring between sites. All 2010 pairwise F_{ST} values for *G. nigra* were significant and ranged from 0.018 between WF1 and EF to 0.039 between MF and GM1 (Table A.2). For *M. fulgida*, 2010 pairwise F_{ST} values were significant for all comparisons between upstream sites (WF1, MF, and GM1) and downstream sites (GM2, GM3, and GM4) and ranged from 0.027 (between MF and GM3) to 0.042 (WF1 and GM2). Comparisons of *M. fulgida* between upstream sites were not significant (e.g., F_{ST} between WF1 and MF = 0.006), nor were comparisons between downstream sites (e.g., GM2 and GM4 = 0.005). Despite having a significant global F_{ST} in 2010, *T. cobitis* had only one significant pairwise F_{ST} value (0.014) between GM1 (upstream) and GM3 (downstream). We found a marginally significant relationship for isolation-by-distance for *M. fulgida* (Mantel r = 0.88, $P = 0.063$) and significant relationship for *T. cobitis* (r = 0.98, $P = 0.037$; Figure 1.3). *Gila nigra* had no correlation between genetic differentiation and stream distance (r = -0.24, $P = 0.743$).

Bayesian analysis of population structure provided evidence for two genetic clusters (K = 2) for each species in the upper Gila River catchment based on 2010 data. *Gila nigra* at MF were genetically distinct from WF1, EF, and GM1 (Figure 1.4), despite having all significant pairwise F_{ST} values. Consistent with pairwise F_{ST} values, *Meda fulgida* had strong support for two genetic clusters; an upstream cluster (WF1, WF2, and MF) and a downstream cluster (GM3, GM4, and GM5). Although global F_{ST} for *T. cobitis* was low, there was weak support for *T. cobitis* having two genetic groups. Individuals at GM1 were weakly differentiated from individuals at downstream sites (GM3, GM4, and GM5) that clustered together; a pattern that was consistent with pairwise F_{ST} values.

Temporal patterns of genetic diversity and structure

All three species exhibited little temporal variation in genetic diversity estimates. For example mean A_R was similar from 2009 to 2010 for *G. nigra* (A_R = 6.6 to 6.4) and *T. cobitis* (A_R = 10.4 to 10.5). The greatest degree of temporal variation in H_E and A_R was observed for *M. fulgida* which decreased slightly across all sites from 2009 (mean H_E = 0.84, A_R = 10.1) to 2010 (H_E = 0.80, A_R = 9.3; Table 1.1). Genetic effective size was consistent between years for *G. nigra* because the 95% CIs overlapped (Table 1.2). Both *M. fulgida* and *T. cobitis* N_e appeared to increase in 2010 from 2009 estimates regardless of estimation method. However the only instance of non-overlapping confidence intervals was for *M. fulgida* N_{eD}. Temporal variation in population genetic structure was consistent between years for *M. fulgida* and *T. cobitis* (Table 1.3, Figure 1.4). The greatest degree of temporal change in structure was observed for *G. nigra* that went from apparent panmixia across two sites in 2009 to significant spatial structuring (F_{ST} = 0.023)

across four sites in 2010. STRUCTURE analysis indicated different patterns of population structure across years, especially between MF and WF2 (Figure 1.4).

Discussion

Evaluating spatial patterns of genetic diversity in a comparative context provides evidence for mechanisms underlying the metapopulation dynamics of each species. Both *M. fulgida* and *T. cobitis* had spatial differentiation patterns reminiscent of isolation-by-distance. Lack of spatial variation in diversity and overall low differentiation of *T. cobitis* suggests this species fits an isolation-by-distance model of gene flow and reflects migration-drift equilibrium within the upper Gila River catchment. Although *M. fulgida* exhibited a positive relationship between distance and F_{ST}, spatial variation in diversity negates migration-drift equilibrium. The pattern of spatial genetic structuring exhibited by *M. fulgida* in conjunction with a gradient of increased diversity downstream has been observed in various stream taxa (Hernandez-Martich and Smith 1997; McGlashan et al. 2001; Mock et al. 2013) for which several non-mutually exclusive mechanisms have been proposed. First, habitat size presumably increases downstream, and thus should harbor numerically larger populations with greater genetic diversity downstream because larger populations are expected to have greater genetic diversity than smaller populations (Frankham et al. 2009). In addition, smaller upstream populations would be subject to greater genetic drift, which could decrease diversity and increase differentiation. Genetic data agree with studies that have documented higher densities of *M. fulgida* in the Cliff-Gila valley (sites GM2 and GM3) than at sites upstream in both the Middle and West forks Gila River (Propst et al. 2008; Whitney et al. 2014). Second, theoretical evidence suggests downstream bias in gene flow (i.e., asymmetric gene flow) could result in

reduced diversity and increased differentiation among upstream populations relative to downstream populations (Morrissey and de Kerckhove 2009). The generally low levels of differentiation, however, preclude any meaningful analysis to estimate asymmetry in gene flow, and therefore, this mechanism cannot be tested with current genetic data. Third, smaller upstream populations could be subject to increased local extinction events. Recolonization by downstream individuals could reduce upstream diversity via founder effects. Nonequilibrium metapopulation processes can increase genetic differentiation expected under migration-drift equilibrium models that do not account for extinction/recolonization events (Whitlock and McCauley 1990). Regardless of the specific mechanisms underlying genetic structure of *M. fulgida* or *T. cobitis*, upstream populations still appear to be genetically connected with downstream populations. The greatest degree of genetic differentiation for *M. fulgida* and *T. cobitis* was observed between upstream headwaters (from GM1 upstream) and downstream mainstem locations (GM2 downstream). Although this is the longest unsampled reach between sample locations, the habitat is primarily canyon-bound (i.e., narrow flood plain with steep canyon walls and high gradient stream channel) and hosts low native fish numbers and high nonnative predator densities (Whitney et al. 2014). However, the relative effect(s) of nonnative predators, hydrologic resistance, and paucity of suitable habitat in this reach to increased differentiation for these species has yet to be investigated.

All three species investigated exhibited relatively low levels of genetic structure (all global F_{ST} values < 0.025) corresponding to moderate to high degree of gene flow, and presumed genetic connectivity among local populations in the upper Gila River catchment. Although low, the reported values of F_{ST} reported here are similar to those

reported for other western cyprinids over similar spatial extent (e.g., Blakney et al. 2014). Tibbets and Dowling (1996) observed greater population structure across tributary drainages for *T. cobitis* than *M. fulgida* and attributed it to *T. cobitis* being more of a habitat specialist and more sedentary than *M. fulgida*. Contrary to presumed dispersal capabilities, *T. cobitis* from the upper Gila catchment displayed less population structure than *M. fulgida*. One possible explanation might be that different mechanisms for population structure act at different spatial scales. For example, lower-gradient, higher-order rivers may pose a greater barrier to gene flow in *T. cobitis* than *M. fulgida* because key habitat features (i.e., cobble riffles) may be scarce or lacking. Similar impediments to small-bodied and benthic freshwater fish species have been postulated as barriers to gene flow in more mesic systems (e.g., Turner and Robison 2006; Hollingsworth and Near 2009). Higher genetic variability among, rather than within catchments, as was observed for *M. fulgida* and *T. cobitis* (Tibbets and Dowling 1996), suggests these species might exhibit the Stream Hierarchy Model of genetic structure (Meffe and Vrijenhoek 1988) at larger spatial scales. Within catchment genetic data from additional populations of these species will be necessary to further test if this model applies to these species.

Gila nigra from 2010 exhibited the highest degree of population structure. For example, significant differentiation was observed for *G. nigra* between sites only 3 km apart (WF1 and MF pairwise $F_{ST} = 0.028$, $P < 0.001$), whereas *M. fulgida* differentiation was negligible between these sites (pairwise $F_{ST} = 0.006$, $P = 0.144$). Yet, the degree of spatial structuring for *G. nigra* was less than that observed for congeneric *G. nigrescens*, Chihuahua chub, over similar spatial extent in the neighboring Mimbres River basin

using a comparable number of microsatellite loci (Osborne at al. 2012). *Gila nigra* also exhibited little spatial variation in diversity and no correlation between F_{ST} and stream distance. Therefore, the Headwater Model of genetic structure (Finn et al. 2007; Hughes et al. 2009) might be an appropriate model for *Gila nigra*. Although originally conceptualized for aquatic taxa with overland dispersal capabilities, the Headwater Model predicts that headwater specialists will only exchange individuals among nearby headwaters. In such a model, lower portions of a watershed, whether altered or unaltered, effectively act as a barrier to gene flow (Hughes et al. 2009). This model for *G. nigra* is supported by high levels of divergence in mitochondrial and nuclear DNA across extant populations of *G. nigra* in its current range (Schwemm 2006).

Typically, observed spatial patterns of genetic structure and diversity are assumed to be stable over time (Tessier and Bernatchez 1999). Consequently, temporal instability or nonequilibrium genetic structure can provide additional information of intrinsic and extrinsic forces affecting metapopulation dynamics (Manel et al. 2003). All three species exhibited little temporal variation in genetic diversity estimates. Contemporary estimates of N_e increased from 2009 to 2010 for all species regardless of estimation method. *Gila nigra* exhibited the most consistent effective size, albeit low, between years and estimation methods because all 95% confidence intervals overlapped. Longer lifespan and delayed sexual maturity of *G. nigra* relative to the other two species might have resulted in the same adults producing 2009 and 2010 offspring and thereby resulting in stability of genetic diversity and N_e estimates. Assessing temporal patterns of diversity for *G. nigra* will require longer time intervals than were available in this study. *Meda fulgida* and *T. cobitis* N_e increased, regardless of method, from 2009 to 2010 (Table 2).

Increased sample size and number of locations sampled in 2010 might account for the observed increase in effective size for these species. However, environmental variability could also account for increased N_e because 2010 had higher springtime flows than 2009 (Whitney et al. 2014). High spring flows are an important component to the natural flow regimes of southwestern streams because they coincide with spawning of native fishes and are positively associated with native fish abundance (Propst et al. 2008; Stefferud et al. 2011; Gido et al. 2013)

Meda fulgida exhibited the greatest temporal stability in genetic structuring compared to the other species (Table 3, Fig. 3 and 4). *Tiaroga cobitis* also exhibited temporal stability in spatial structuring between years, except that the degree of structuring between upstream and downstream sites became more pronounced (Fig. 4), presumably because of increased sample size in 2010. Both species displayed similar patterns of genetic structure versus stream distance between years (Fig. 3) suggesting temporal stability in gene flow during this study. Despite temporal stability in diversity estimates, *G. nigra* exhibited temporal instability in population structure. For example, WF2 and MF were not significantly differentiated in 2009 but were in 2010. Although one could argue that such differences in genetic structuring between years could be an artifact of increased geographical extent of our sampling effort in 2010, biologically meaningful temporal changes in genetic structuring have been observed in other aquatic species (Crispo and Chapman 2010; McElroy et al. 2011; Apodaca et al. 2013). Natural disturbances have been documented to alter population genetic structure via mixing of individuals from distinct populations in limited deep-water refugia during drought (McElroy et al. 2011) or large-scale displacement of individuals (Apodaca et al. 2013).

High spring flows in 2010 might have allowed greater movement of *G. nigra* relative to 2009 thus altering genetic structure. These same flows positively affected reproductive success of *G. nigra* because the species was more common in 2010 than 2009.

Genetic effective size determines the degree to which evolutionary forces such as genetic drift, selection, and migration act on a population, and as such, is an important parameter for conservation genetics. All species investigated here had genetic effective sizes in the range of 60 to 600 which is below the threshold, $N_e < 1000$, considered adequate to maintain long-term genetic variability (Frankham et al. 2014). *Tiaroga cobitis* sampled in 2010 had the largest N_{eD} of all species but this estimate also had a large 95% CI. Low precision is expected for populations with large N_e because the methods employed here have difficulty obtaining reliable estimates for large populations (Waples and Do 2010). The apparent significant increase of N_{eD} for *M. fulgida* was most likely a result of increased number of individuals making up the 2010 sample as LDNE is particularly sensitive to differences in sample size (Waples and Do 2008, 2010). *Gila nigra* exhibited the lowest values of N_e and gene diversity among Gila River fishes but these values were consistent with those from threatened *G. nigrescens* in the neighboring Mimbres River (Osborne et al. 2012). Extremely small N_e values (< 100) are of particular concern because populations can accumulate deleterious mutations leading to mutational meltdown (Higgins and Lynch 2001). Relatively low N_e in all species suggests random genetic drift could be the dominant force shaping each species' evolutionary trajectory. Indeed, genetic drift was identified as being a major force in reduced diversity of major histocompatibility complex (MHC) alleles and microsatellites in Gila trout, *Oncorhynchus gilae* (Peters and Turner 2008). Although theory indicates

that drift is a dominant evolutionary force in small populations, recent empirical studies have documented greater putative adaptive differentiation among small populations, suggesting natural selection can affect small populations in addition to drift (Fraser et al. 2014). Therefore, further evaluation of the adaptive potential of Gila River fishes is warranted.

Conservation implications

The comparatively pristine nature of the system has enabled it to be one of the last remaining strongholds for *G. nigra*, *M. fulgida*, and *T. cobitis.* Security of these populations is compromised however by the presence of nonnative fishes, extended drought, and large-scale disturbances. Large nonnative piscivores, such as flathead catfish *Pylodictis olivaris* and smallmouth bass *Micropterus dolomieu* are present in the catchment and *P. olivaris* has expanded its range by recently colonizing lower reaches of the West, Middle, and East Forks (Propst et al. 2014), overlapping the current distribution of *G. nigra*. Continued numerical suppression of *G. nigra* by nonnatives could exacerbate already low N_e to the point of entering the extinction vortex (Gilpin and Soulé 1986). Our genetic analyses indicated that upstream populations of *M. fulgida* and *T. cobitis* likely rely on downstream populations for augmentation and maintaining genetic diversity. *Gila nigra* lacks a similar source for augmentation because the only other known population within the upper Gila catchment occurs in Turkey Creek (Fig. 2) but the degree to which these individuals move into the mainstem and upstream is uncertain. Reaches with high nonnative predator loads, such as the canyon-bound reach separating the upstream and downstream populations of *M. fulgida* and *T. cobitis*, could inhibit dispersal of fishes (Fraser et al. 1995; Harvey et al. 2004) reducing gene flow between

these populations or isolating them completely. Furthermore, proposed diversion structures as part of the Arizona Water Settlement Act (Fig. 2) could further threaten native Gila River fishes by increasing fragmentation and altering the natural flow regime. These threats could be exacerbated by disturbances, such as extended drought and ash-debris flows following wildfires (Whitney et al. in press). Management actions should focus on the entire riverscape and strive to maintain natural ecosystem resilience. For example, activities that maintain or restore structural and functional connectivity (sensu Kindlmann and Burel 2008) and targeted nonnative removal (Propst et al. 2014) are likely to benefit all native species of the upper Gila River basin. In addition, continued genetic monitoring of listed species will be necessary to ensure no further erosion of genetic diversity occurs and to evaluate the efficacy of management practices.

Conclusion

As with many imperiled species, Gila River fishes have suffered extensive range declines and decreased population sizes (Propst 1999; Propst et al. 2008). The species we focused on here still maintain populations elsewhere in the greater Gila River drainage, but each is now isolated demographically and genetically from the upper Gila catchment in New Mexico. In addition, populations of these species outside of the upper Gila catchment occupy smaller and more fragmented systems. Characterization of patterns of genetic diversity, gene flow, and genetic drift of imperiled species in comparatively unaltered systems provides information that can aid in management activities for species occupying fragmented systems including augmentation, habitat restoration, and repatriations. Knowledge of relatively natural genetic patterns is necessary for restoring evolutionarily important metapopulation dynamics for repatriated populations.

Incorporation of spatial metapopulation processes in species recovery plans is critical for decisions regarding which habitats and the quantity of habitat to restore (Huxel and Hastings 1999) and could mitigate the effects of habitat loss and fragmentation (Lewis et al. 1996).

Literature Cited

Allan JD, Flecker AS (1993) Biodiversity conservation in running waters. Bioscience 43: 32-43

Apodaca JJ, Trexler JC, Jue NK, Schrader M, Travis J (2013) Large-Scale Natural Disturbance Alters Genetic Population Structure of the Sailfin Molly, *Poecilia latipinna*. American Naturalist 181: 254-263

Blakney JR, Loxterman JL, Keeley ER (2014) Range-wide comparisons of nothern leatherside chub populations reveal historical and comtemporary patterns of genetic variation. Conservation Genetics 15: 757-770

Carlson CA, Muth RT (1989) The Colorado River: lifeline of the American Southwest. Canadian Special Publication of Fisheries and Aquatic Sciences 106: 220-239

Crispo E, Chapman LJ (2010) Temporal variation in population genetic structure of a riverine African cichlid fish. Journal of Heredity 101: 97-106

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359-361

England PR, Cornuet JM, Berthier P, Tallmon DA, Luikart G (2006) Estimating effective population size from linkage disequilibrium: severe bias in small samples. Conservation Genetics 7: 303-308

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1: 47-50

Fagan WF (2002) Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83: 3243-3249

Fagan WF, Unmack PJ, Burgess C, Minckley WL (2002) Rarity, fragmentation, and extinction risk in desert fishes. Ecology 83: 3250-3256

Finn DS, Blouin MS, Lytle DA (2007) Population genetic structure reveals terrestrial affinities for a headwater stream insect. Freshwater Biology 52: 1881-1897

Fradkin PL (1981) A river no more: the Colorado River and the West. University of Arizona Press, Tucson, Arizona, USA.

Frankham R (2005) Genetics and extinction. Biological Conservation 126: 131-140

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to Conservation Genetics, Second edn. Cambridge University Press, New York

Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170: 56-63

Fraser DF, Gilliam JF, Yiphoi T (1995) Predation as an agent of population fragmentation in a tropical watershed. Ecology 76: 1461-1472

Fraser DJ, Debes PV, Bernatchez L, Hutchings JA (2014) Population size, habitat fragmentation, and the nature of adaptive variation in a stream fish. Proceedings of the Royal Society B-Biological Sciences. DOI: 10.1098/rspb.2014.0370

Gido KB, Propst DL, Olden JD, Bestgen KR (2013) Multidecadal responses of native and introduced fishes to natural and altered flow regimes in the American Southwest. Canadian Journal of Fisheries and Aquatic Sciences 70: 554-564

Gilpin ME, Soulé ME (1986) Minimum viable populations: Processes of species extinction. In: Conservation Biology: The Science of Scarcity and Diversity (ed. Soulé ME), pp. 19-34. Sinauer Associates, Inc., Sunderland, MA, USA

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486

Harvey BC, White JL, Nakamoto RJ (2004) An emergent multiple predator effect may enhance biotic resistance in a stream fish assemblage. Ecology 85: 127-133

Hernandez-Martich JD, Smith MH (1997) Downstream gene flow and genetic structure of *Gambusia holbrooki* (eastern mosquitofish) populations. Heredity 79: 295-301

Higgins K, Lynch M (2001) Metapopulation extinction caused by mutation accumulation. PNAS 98: 2928-2933

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. Genetical Research 38: 209-216

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: Sequencing and cloning. In: Molecular systematics, 2nd edn. Sinauer Associates, Sunderland.

Hollingsworth PR, Near TJ (2009) Temporal patterns of diversification and microendemism in eastern highland endemic barcheek darters (Percidae: Etheostomatinae). Evolution 63: 228-243

Holm S (1979) A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6: 65-70

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9: 1322-1332

Hughes JM, Schmidt DJ, Finn DS (2009) Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. Bioscience 59: 573-583

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53: 1898-1914

Huxel GR, Hastings A (1999) Habitat loss, fragmentation, and restoration. Restoration Ecology 7: 309-315

Kindlmann P, Burel F (2008) Connectivity measures: a review. Landscape Ecology 23: 879-890

Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. Molecular Ecology 11: 2445-2449

Lewis CA, Lester NP, Bradshaw AD, Fitzgibbon JE, Fuller K, Hakanson L, Richards C (1996) Considerations of scale in habitat conservation and restoration. Canadian Journal of Fisheries and Aquatic Sciences 53: 440-445

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18: 189-197

McElroy TC, Kandl KL, Trexler JC (2011) Temporal Population Genetic Structure of Eastern Mosquitofish in a Dynamic Aquatic Landscape. Journal of Heredity 102: 678-687

McGlashan DJ, Hughes JM, Bunn SE (2001) Within-drainage population genetic structure of the freshwater fish Pseudomugil signifer (Pseudomugilidae) in northern Australia. Canadian Journal of Fisheries and Aquatic Sciences 58: 1842-1852

Meffe GK, Vrijenhoek RC (1988) Conservation genetics in the management of desert fishes. Conservation Biology 2: 157-169

Minckley WL, DeMarais BD (2000) Taxonomy of chubs (Teleostei, Cyprinidae, Genus *Gila*) in the American Southwest with comments on conservation. Copeia 2000: 251-256

Mock KE, Box JCB, Chong JP, Furnish J, Howard JK (2013) Comparison of population genetic patterns in two widespread freshwater mussels with contrasting life histories in western North America. Molecular Ecology 22: 6060-6073

Morrissey MB, de Kerckhove DT (2009) The maintenance of genetic variation due to asymmetric gene flow in dendritic metapopulations. American Naturalist 174: 875-889

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

New Mexico Department of Game and Fish (2006) Colorado River basin chubs (roundtail chub *Gila robusta*, Gila chub *Gila intermedia*, headwater chub *Gila nigra*) recovery plan. NMDGF Conservation Services Division, Santa Fe, New

Mexico, USA. http://www.wildlife.state.nm.us/conservation/documents/ChubsRecoveryPlan.pdf

Osborne M, Sharp A, Monzingo J, Propst DL, Turner TF (2012) Genetic analysis suggests high conservation value of peripheral populations of Chihuahua chub (*Gila nigrescens*). Conservation Genetics 13: 1317-1328

Paroz Y, Propst D, Stefferud J (2006) Long-term monitoring of fish assemblages in the Gila River drainage, New Mexico. Technical Report. New Mexico Department of Game and Fish, Santa Fe, New Mexico

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539

Peters MB, Turner TF (2008) Genetic variation of the major histocompatibility complex (MHC class II beta gene) in the threatened Gila trout, *Oncorhynchus gilae gilae*. Conservation Genetics 9: 257-270

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12: 844-855

Pilger TJ, Gido KB, Propst DL (2010) Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: implications for native fish conservation. Ecology of Freshwater Fish 19: 300-321

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959

Propst DL (1999) Threatened and endangered fishes of New Mexico. Technical Report No. 1. New Mexico Department of Game and Fish, Santa Fe, New Mexico

Propst DL, Gido KB, Stefferud JA (2008) Natural flow regimes, nonnative fishes, and native fish persistence in arid-land river systems. Ecological Applications 18: 1236-1252

Propst DL, Gido KB, Whitney JE, Gilbert EI, Pilger TJ, Monié AM, Paroz YM, Wick JM, Monzingo JA, Myers DM (2014) Efficacy of mechanically removing nonnative predators from a desert stream. River Research and Applications. DOI: 10.1002/rra.2768

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43: 258-275

R Core Team (2012) R: A language and environment for statistical computing. Vienna, Austria. www.r-project.org

Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Richter BD, Braun DP, Mendelson MA, Master LL (1997) Threats to imperiled freshwater fauna. Conservation Biology 11: 1081-1093

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145: 1219-1228

Rousset F (2008) GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8: 103-106

Schwemm MR (2006) Genetic variation in the *Gila robusta* complex (Teleostei: Cyprinidae) in the Lower Colorado River. Dissertation, Arizona State University

Seager R, Ting MF, Held I, Kushnir Y, Lu J, Vecchi G, Huang HP, Harnik N, Leetmaa A, Lau NC, Li CH, Velez J, Naik N (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316: 1181-1184

Slatkin M (1985) Gene flow in natural populations. Annual Review of Ecology and Systematics 16: 393-430

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Stefferud JA, Gido KB, Propst DL (2011) Spatially variable response of native fish assemblages to discharge, predators and habitat characteristics in an arid-land river. Freshwater Biology 56: 1403-1416

Strayer DL, Dudgeon D (2010) Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society 29: 344-358

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). Molecular Ecology 8: 169-179

Tibbets CA, Dowling TE (1996) Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). Evolution 50: 1280-1292

Trujillo, JD, Pilger TJ, Douglas MR, Douglas ME, Turner TF (2012). Microsatellite markers for Longfin Dace, *Agosia chrysogaster*, a sentinel fish species in

imperiled arid-land rivers of Sonoran Desert. Conservation Genetics Resources 4: 927-929

Turner TF, Robison HW (2006) Genetic diversity of the caddo madtom, Noturus taylori, with comments on factors that promote genetic divergence in fishes endemic to the ouachita highlands. Southwestern Naturalist 51: 338-345

U.S. Fish and Wildlife Service (2006) Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To List a Distinct Population Segment of the Roundtail Chub in the Lower Colorado River Basin and To List the Headwater Chub as Endangered or Threatened With Critical Habitat. Federal Register 71: 26007-26017

U.S. Fish and Wildlife Service (2012) Endangered and Threatened Wildlife and Plants; Endangered Status and Designations of Critical Habitat for Spikedace and Loach Minnow: Final rule. Federal Register 77: 10810-10932

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538

Vorosmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM (2010) Global threats to human water security and river biodiversity. Nature 467: 555-561

Wang JL (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Molecular Ecology 18: 2148-2164

Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity 89: 438-450

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics 7: 167-184.

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8: 753-756

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3: 244-262

Waples RS, Waples RK (2011) Inbreeding effective population size and parentage analysis without parents. Molecular Ecology Resources 11: 162-171

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western US forest wildfire activity. Science 313: 940-943

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution 44: 1717-1724

Whitney JE, Gido KB, Propst DL (2014) Factors associated with the success of native and nonnative species in an unfragmented arid-land riverscape. Canadian Journal of Fisheries and Aquatic Science, 71: 1134-1145

Whitney JE, Gido KB, Pilger TJ, Propst DL, Turner TF (2015) Metapopulation analysis indicates native and nonnative-fishes respond differently to effects of wildfire on desert streams. Ecology of Freshwater Fish. DOI: 10.1111/eff.12217

Wofford JEB, Gresswell RE, Banks MA (2005) Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecological Applications 15: 628-637

Wright S (1931) Evolution in Mendelian populations. Genetics 16: 97-159.

Tables and Figures

Table 1.1 Genetic summary statistics for three protected fishes of the upper Gila River catchment sampled from 2009 and 2010. At each site, sample size (n), gene diversity (H_E), observed heterozygosity (H_O), allelic richness (A_R), inbreeding coefficient (F_{IS}), and mean Queller and Goodnight (1989) estimator of relatedness (r) are reported. Missing values (indicated with a dash) were not estimated due to small sample size. Site codes correspond to sites in Fig. 1

(On next page)

Species Year	Site	n	H_E	H_O	A_R[a]	F_{IS}[b]	r[b]
Gila nigra							
2009	MF	29	0.655	0.617	6.59	**0.059**	0.017
	WF2	40	0.658	0.599	6.70	**0.092**	**0.033**
2010	WF1	26	0.687	0.656	6.69	0.047	-0.049
	MF	20	0.641	0.595	5.84	**0.074**	**0.061**
	WF2	3	0.623	0.617	-	-	-
	EF	19	0.670	0.657	6.80	0.021	0.026
	GM1	12	0.664	0.663	6.14	0.001	0.040
Meda fulgida							
2009	WF2	33	0.799	0.788	9.66	0.013	**0.065**
	GM2	32	0.874	0.811	13.93	**0.073**	-0.033
	GM3	28	0.850	0.823	11.03	0.033	-0.007
2010	WF1	30	0.755	0.657	8.22	**0.132**	**0.102**
	MF	34	0.788	0.699	8.43	**0.115**	**0.054**
	WF2	30	0.761	0.688	7.84	**0.098**	**0.088**
	GM2	28	0.841	0.802	10.37	**0.048**	0.002
	GM3	31	0.846	0.773	10.58	**0.087**	-0.010
	GM4	17	0.836	0.784	10.63	**0.065**	-0.005
Tiaroga cobitis							
2009	GM1	21	0.780	0.702	10.00	**0.102**	**-0.049**
	GM2	30	0.707	0.687	10.49	0.028	**0.029**
	GM3	22	0.729	0.700	9.95	0.041	0.007
2010	GM1	30	0.725	0.644	9.95	**0.115**	0.021
	GM2	29	0.739	0.727	11.04	0.017	-0.004
	GM3	34	0.716	0.643	10.76	**0.103**	**0.027**
	GM4	19	0.741	0.694	10.15	**0.065**	-0.008

[a] Allelic richness based on sample size of: 11 for *G. nigra*, 14 for *M. fulgida*, and 18 for *T. cobitis*.
[b] Values in bold font indicate significantly different from zero at $\alpha = 0.05$ level

Table 1.2 Genetic effective size estimated using the linkage disequilibrium method (N_{eD}, Waples and Do 2008) and sibship method (N_{eS}, Wang 2009) for three protected fishes of the upper Gila River catchment in 2009 and 2010

	N_{eD} (95% CI)		N_{eS} (95% CI)	
Species	2009	2010	2009	2010
Gila nigra	80 (61-112)	105 (78-151)	60 (41-89)	83 (60-117)
Meda fulgida	158 (120-222)	325 (244-470)	109 (78-155)	167 (128-220)
Tiaroga cobitis	157 (93-397)	602 (292-20719)	100 (70-147)	156 (116-211)

Table 1.3 Population level F_{ST} values for three protected fishes of the upper Gila River catchment sampled during 2009 and 2010

	2009	2010
Species	F_{ST} (95 % CI)	F_{ST} (95 % CI)
Gila nigra	0.008 (-0.001-0.018)	0.028 (0.015-0.040)
Meda fulgida	0.022 (0.015-0.028)	0.021 (0.015-0.026)
Tiaroga cobitis	0.012 (-0.001-0.030)	0.008 (0.005-0.011)

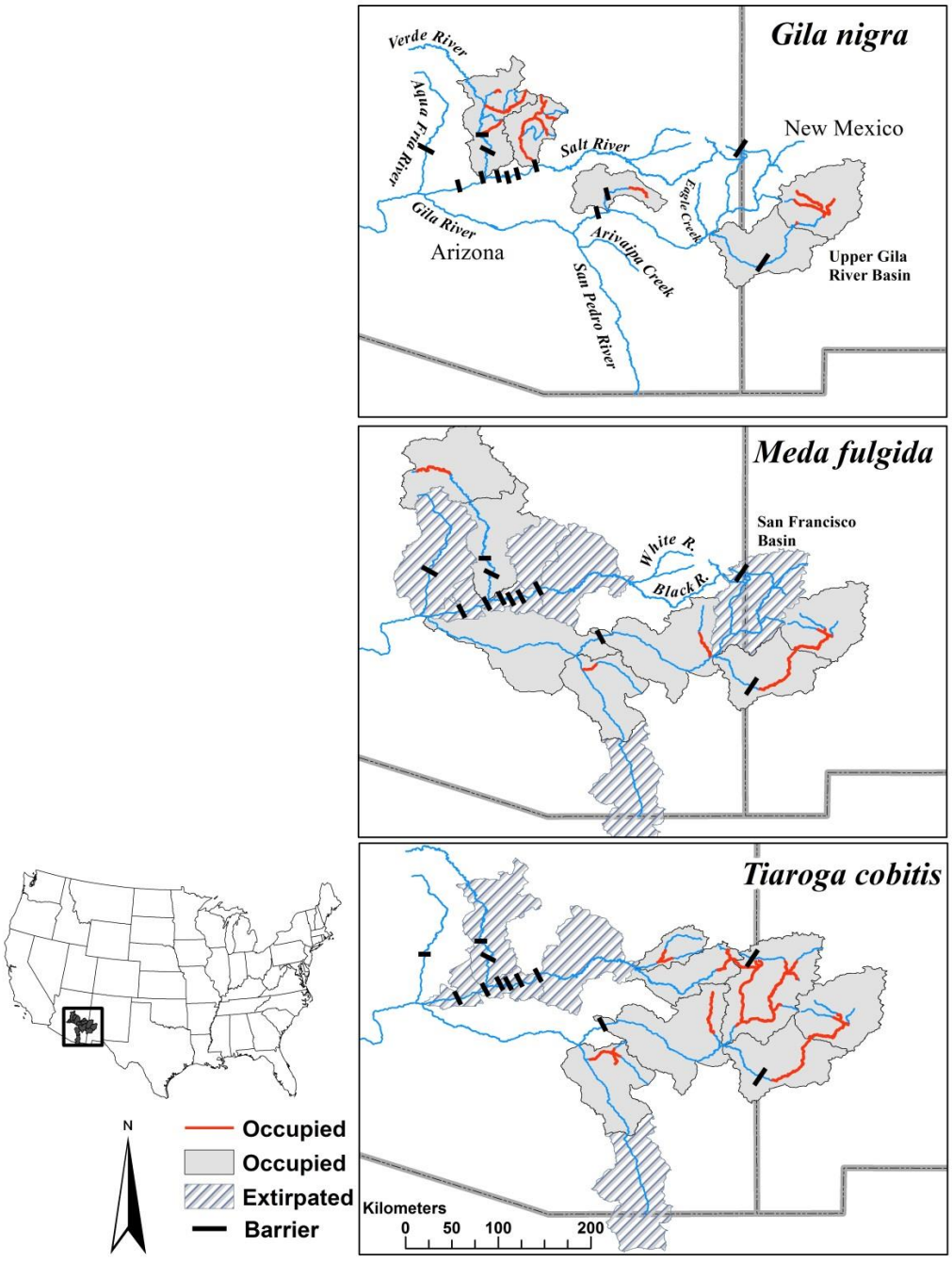

Figure 1.1 Range maps for three protected fishes endemic to the Gila River basin of New Mexico and Arizona, USA, indicating historical and current distributions in 8-digit USGS hydrologic unit code (HUC) watersheds. Streams currently occupied are indicated in red. Data on current and historical distributions from NatureServe (www.natureserve.org) and U.S. Bureau of Reclamation (http://www.usbr.gov/lc/phoenix/biology/azfish/profintro.html)

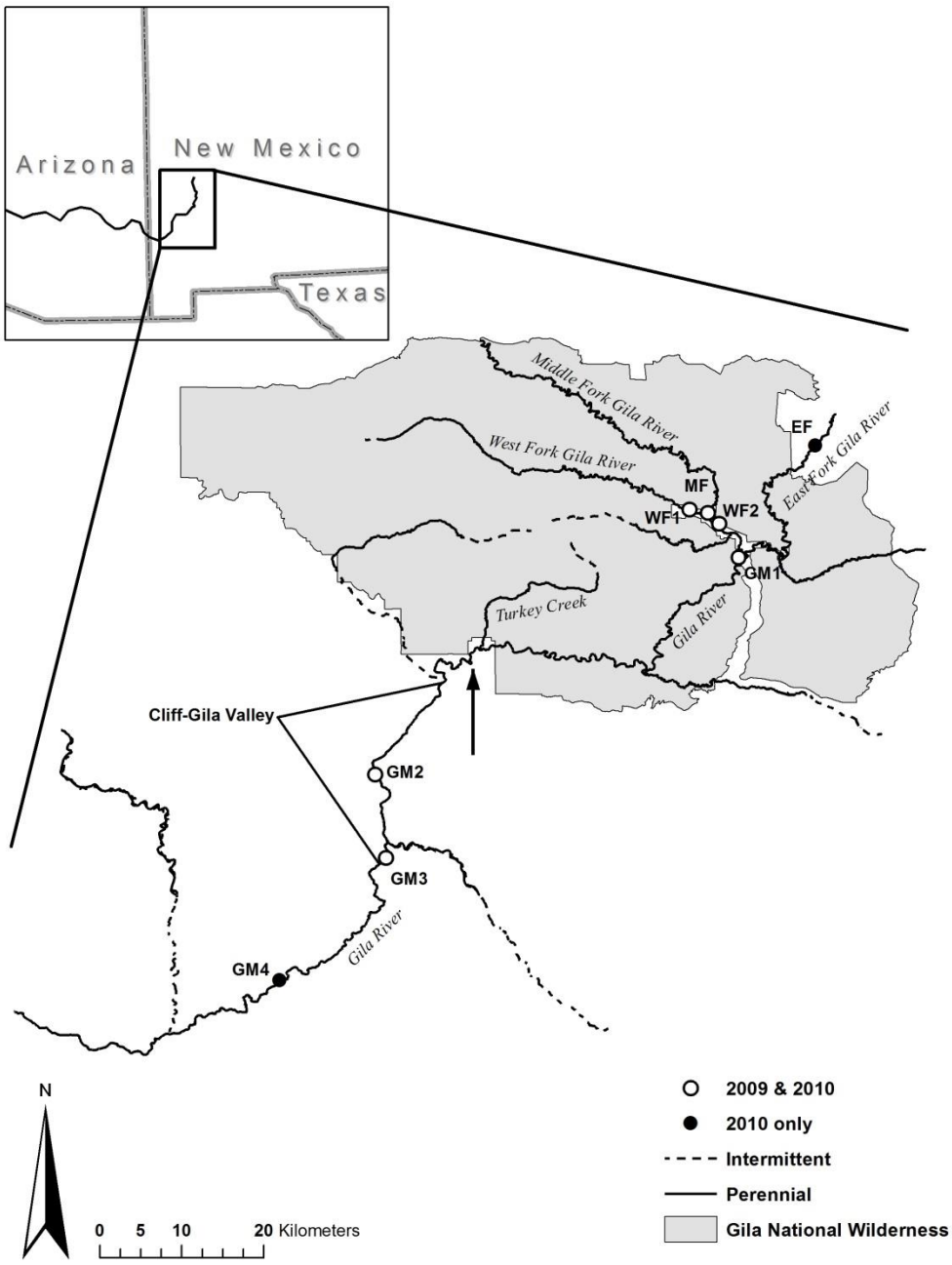

Figure 1.2 Sample sites for three protected fishes of the upper Gila River catchment, New Mexico, USA. Samples collected from locations in 2009 are indicated with open circles and additional locations sampled in 2010 indicated by closed circles. Large arrow represents the approximate location of a proposed diversion structure (see text). Site numbers correspond to the site names in Table 2

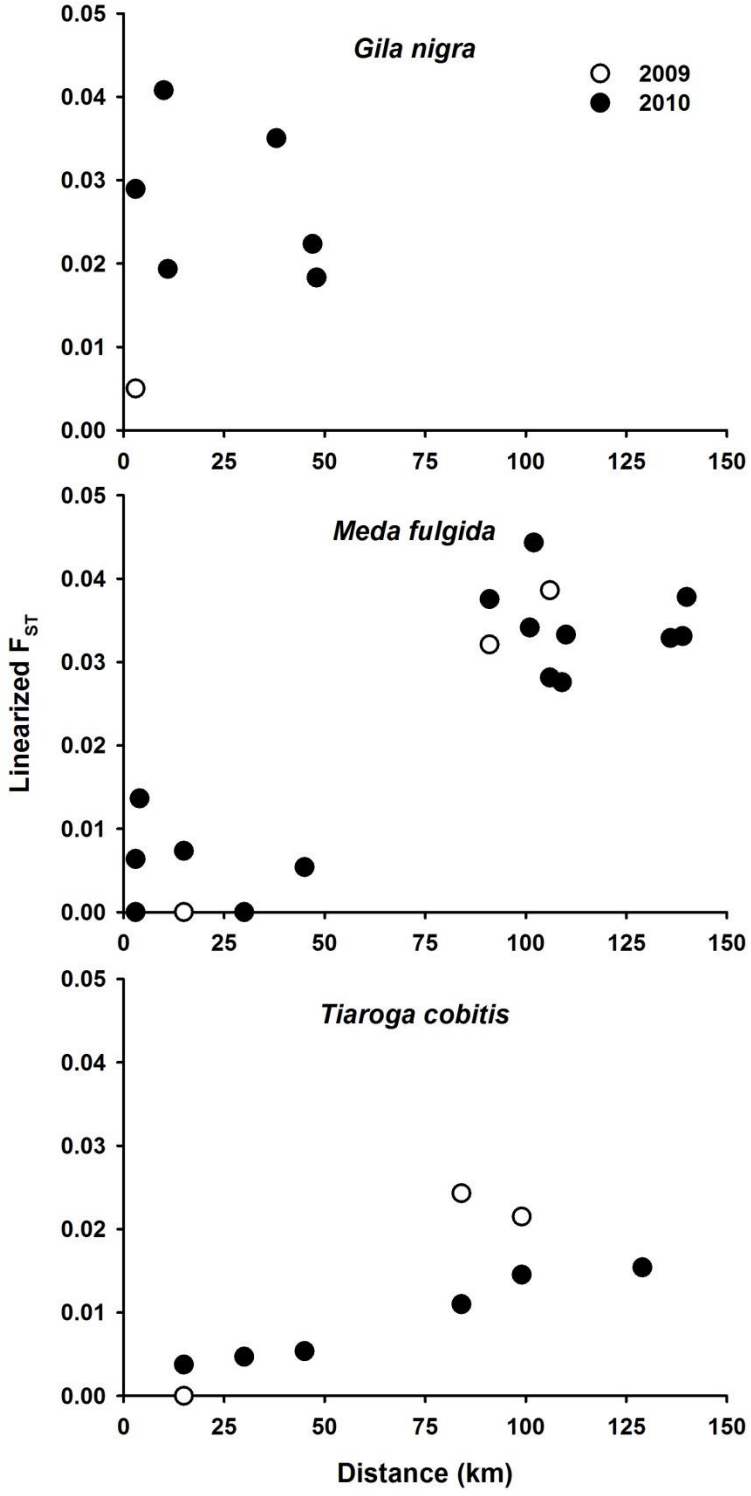

Figure 1.3 Biplots of pairwise linearized F_{ST} versus stream distance in kilometers for three protected fishes of the upper Gila River catchment, New Mexico, USA. Samples collected in 2009 indicated by open circles and 2010 by closed circles

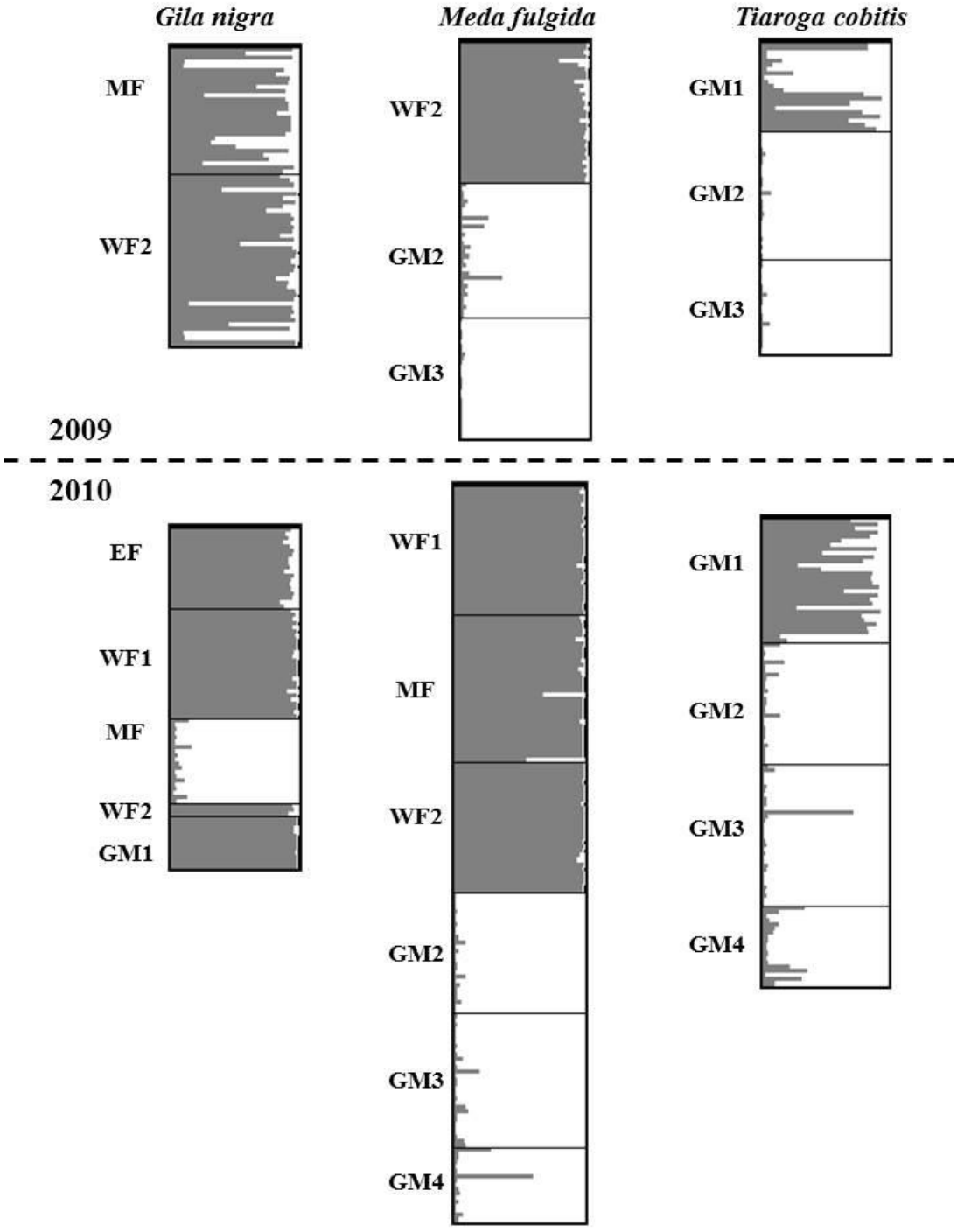

Figure 1.4 Assignment probability plots obtained from STRUCTURE for three protected fishes of the upper Gila River catchment sampled in 2009 (above dashed line) and 2010 (below). Each horizontal bar represents an individual and the probability of being assigned to one of two genetic units (K = 2, represented by either gray or white). Site abbreviations correspond to Table 1 and Fig. 2

Chapter 2

EMERGENT PATTERNS OF POPULATION GENETIC STRUCTURE FOR AN ARID LAND FISH COMMUNITY

Abstract

Extrinsic factors are known to elicit similar patterns of genetic variation across species with intrinsic differences. Yet explaining the prevalence of species-specific genetic patterns requires evaluation of how intrinsic traits unique to groups of species with similar life histories (i.e., traits related to dispersal, and thus, gene flow) interact with extrinsic factors. We used microsatellite DNA loci to characterize species-specific genetic patterns across nine distantly related fishes comprising the fish community of the upper Gila River catchment, New Mexico, USA. Whereas, we expected aspects of the riverscape (distance between populations and number of confluences) to elicit shared responses of genetic structure across species, we expected species-specific responses to be related to life-history traits influencing dispersal. Species exhibited a wide variety of spatial structuring, including (near) panmixia, isolation-by-distance, regional structuring and patterns related to differences in local genetic effective size (N_e). Whereas the overall degree of genetic differentiation (F_{ST}) among sample locations was not strongly predicted by species traits, patterns of genetic structuring on the landscape via distance (Mantel's *r*) was strongly associated with variation of fecundity across species. We observed that distance, as a mechanism for isolation, has a stronger effect on less fecund species than highly fecund species. Such differential responses of species to landscape features

indicates management actions might have different consequences for maintaining species evolutionary potential.

Introduction

Genetic differentiation (i.e., differences in allele frequencies) among subdivided populations is a direct result of interrupted gene flow (Wright 1931; Slatkin 1987) and a precursor to speciation (Coyne & Orr 2004). The degree of differentiation among populations depends on a balance of microevolutionary forces (i.e., genetic drift, gene flow, mutation, and natural selection), the magnitudes of which are primarily determined by genetic effective size. However, mediation of gene flow by ecological and environmental factors can produce emergent patterns of genetic variation that obfuscate the specific mechanisms responsible for differentiation of allelic frequencies (Avise 2000). Identification of key factors related to gene flow is a necessary step in understanding the evolutionary trajectory of species and for making generalizations across species with different ecologies. Furthermore, knowledge of specific traits related to genetic patterns will allow better predictions for species-specific responses to changing landscapes (e.g., Blanchet et al. 2010).

The role of extrinsic factors, principally geography, in shaping patterns of genetic variation has been relatively well studied over a broad range of spatial and temporal scales. Phylogeographic studies provide evidence that large-scale landscape features and historical physiographic events can produce similar patterns of genetic variation, such as genetic breaks, across broad taxonomic groups (e.g., Avise 1992; Meirmans et al. 2011). At smaller scales, landscape genetic analyses have addressed how spatial habitat

heterogeneity and fragmentation influence patterns of gene flow across species (Manel et al. 2003; Manel & Holderegger 2013). Such comparative analyses of sympatric species have highlighted the role of extrinsic properties for shaping genetic variation while revealing other patterns related to intrinsic differences among species (e.g., Turner & Trexler 1998; Manier & Arnold 2006). The prevalence of species-specific structuring across biological systems, due to interactions between life history traits and landscape factors, has resulted in few generalizations regarding the role intrinsic life history traits play in moderating microevolutionary processes (Manel et al. 2003).

Given the importance of gene flow to differentiation and evolutionary trajectories of species, it is no surprise researchers have focused on dispersal-related traits to explain varying levels of differentiation among species. Population genetic theory suggests an inverse relationship between species' dispersal capabilities and genetic differentiation (Wright 1931; Slatkin 1987). However, empirical studies have been equivocal in the degree to which dispersal-related traits affect genetic structure, in part because of a disconnect in time scales over which genetic estimates of differentiation apply and ecological measures of dispersal occur (reviewed by Bohonak 1999). Also, large sample sizes are generally required to detect subtle patterns in noisy comparative datasets (Riginos et al. 2011). Nevertheless, examples of dispersal and life history traits influencing genetic differentiation continue to be found across biological systems including seed type and pollination mode in plants (Givnish 2010; Meirmans et al. 2011), pelagic larval duration in marine invertebrates and fishes (Riginos et al. 2011; Faurby & Barber 2012), fecundity, egg size, and dispersal ability in freshwater fishes (Turner & Trexler 1998; Gomez-Uchida et al. 2009), and habitat specificity in freshwater fish

(Tibbets & Dowling 1996) and birds (Burney & Brumfield 2009). Furthermore, comparisons of sympatric species have revealed contrasting patterns of genetic structure, emphasizing the interaction between life history and landscape in shaping genetic differentiation (e.g., Turner & Trexler 1998; Gomez-Uchida et al. 2009). The interaction between intrinsic and extrinsic factors has remained understudied and thus few generalizations exist regarding species-specific traits leading to different genetic patterns on a shared landscape.

Freshwater fish communities are ideal for evaluating the influence of life history traits on genetic structuring because the linear nature of the habitat network simplifies ecological and genetic models that describe evolutionary dynamics (Rodriguez 2002; Koizumi et al. 2006). Because dispersal and gene flow are constrained by the stream network, landscape factors are reduced to distance and in-stream barriers to gene flow. Furthermore, directionality in water flow, elevation, and habitat size inherent to riverscapes as well as instream barriers provide opportunities for evaluating asymmetries in genetic connectivity among populations (Morrissey & de Kerckhove 2009). Dispersal of most stream fishes occurs over relatively short distances with infrequent long-distance dispersal events (Skalski & Gilliam 2000, 2003; Rodriguez 2010). This residency behavior may contribute to genetic differentiation among spawning aggregates in different tributaries and subcatchments within a drainage basin. A number of conceptual models hypothesize the effect of dispersal and life history on patterns of gene flow within a stream network (reviews by Hughes et al. 2009; 2013). For example, hierarchical spatial structuring is expected for species occurring in multiple drainages because individuals in tributaries within a catchment should be more closely related than among

individuals across tributary catchments (Meffe & Vrijenhoek 1988; Hughes et al. 2009), yet the specific dispersal or life history traits responsible for these patterns remain unclear. In addition, the emergence of "chaotic" genetic structure (Johnson & Black 1984; Hedgecock & Pudovkin 2011) unrelated to stream network architecture can result from metapopulation processes such as extirpation and recolonization (Whitlock & McCauley 1990) or disturbance events (Apodaca et al. 2013; Banks et al. 2013). Additionally, strong genetic drift due to low genetic effective size (N_e) could lead to genetic structuring unrelated to network architecture. Therefore, comparative analyses using multiple species in a shared riverscape could help to distinguish between extrinsic (patterns similar across species with intrinsic differences) and intrinsic causes (species-specific patterns related to intrinsic differences) of genetic variation (Manel et al. 2003).

Here we used a combination of landscape genetics and multi-species comparisons to evaluate the influence of life-history traits on gene flow and genetic structuring in a stream fish community. Although meta-analyses including hundreds of species can provide greater power to discern which traits are responsible for observed genetic patterns, they often cannot account for geographic factors because focal species are not co-distributed (Riginos et al. 2011). Comparative studies of sympatric species using a common sampling regime can help control for shared geographic influences, but often for only a small number of species. We collected genetic data from nine co-occurring species from within the same riverscape to control for environmental factors and shared historical influences among species (e.g.,Tibbets & Dowling 1996). Differences in observed genetic patterns across species should thus be a result of species-specific dispersal and life-history related traits. Our goal was to provide insight about mechanisms influencing

population structuring in stream networks by comparing life history and genetic data across distantly related, but broadly co-occurring species including native and introduced species. Specifically, our research objectives were to 1) quantify spatial genetic variation for multiple species of a stream fish community, 2) characterize the role of landscape and genetic drift on population structure of each species using landscape genetic techniques, and 3) combine results of genetic analyses with ecological data on dispersal, life history, and range size to evaluate if relevant interactions exist between intrinsic and extrinsic factors.

Methods

Study Site

Our study took place on the upper Gila River basin in southwestern New Mexico, USA (Figure 2.1) .The upper Gila River is ideal for studying natural levels of gene flow because there are no major human-engineered barriers likely to influence dispersal (except see below) and a quasi-natural flow regime persists (Propst et al. 2008). We selected 16 sample locations representing > 200 rkm (river kilometers) with an elevation gradient of nearly 900 m (1161 to 2059 m above sea level). Land use changes along the course of the Gila River result in a transition of low- to moderate-habitat alteration. Upstream tributaries, including West, Middle, and East Forks of the Gila River are encompassed by Gila National Wilderness Area and are relatively pristine. The mainstem Gila River (flowing downstream from the confluence of West and East Forks) flows through a high gradient canyon-bound reach surrounded by wilderness area followed by a lower gradient valley with sparse human settlement. Water diversion (via temporary

earthen structure) for irrigation in the valley contributes to reduced discharge through the valley but the absence of a permanent diversion structure was not expected to affect dispersal. Leaving the valley, the Gila River flows through a second canyon-bound reach and into a second valley reach with increased modification and in which whole-channel drying has been observed. The most downstream site was located just below a permanent diversion structure (height ~ 3 m). Collections of individuals above this diversion would have been preferable but was not feasible due to complete desiccation at sampling time. Several tributary sites also experience reduced structural connectivity from the mainstem by dry reaches during summer months, particularly Blue Creek, Black Canyon and Little Creek. All tributary locations are high gradient streams surrounded by mountainous landscapes. Downstream of our lowest sample location, the Gila River is frequently dry and thus we consider immigration of individuals from populations in Arizona to be infrequent. Hence, populations of each species in the upper Gila River were considered closed to migration from the lower Gila River.

Fishes of the upper Gila River basin comprise a nearly intact native fish community consisting of nine endemic or near-endemic species, and 12 nonnative fishes. We targeted warm water native fishes including *Agosia chrysogaster* (longfin dace), *Gila nigra* (headwater chub), *Meda fulgida* (spikedace), *Rhinichthys osculus* (speckled dace), *Tiaroga cobitis* (loach minnow), *Catostomus insignis* (Sonora sucker), and *Pantosteus clarkii* (desert sucker). Two commonly occurring nonnative species were also targeted; *Ameiurus natalis* (yellow bullhead catfish) and *Micropterus dolomieu* (smallmouth bass) because they are known predators in the system (Pilger et al. 2010) and exhibit divergent life-history strategies from native species (Olden et al. 2006). Given the variation in body

size and life history among focal species (propensity for dispersal, body size, egg size; Table 2.1) we predicted that species would display different spatial patterns of genetic structuring and distribution of genetic diversity.

Sampling

We sampled each location from October to November of 2010 for native and nonnative species using a combination of backpack electrofishing and seining (4.6 x 1.2m, 3.2mm mesh). During each sampling event, individuals of similar size, typically age 1, for each species were collected in an attempt to include only individuals of the same cohort. Tissue samples for DNA extraction were collected by clipping a small portion (< 5 mm^2) of the caudal fin and preserving it in 95% ethanol. Sampled individuals were returned to the stream at the capture site.

Microsatellite Genotyping

Genomic DNA was extracted from air-dried fin clips using standard proteinase-K digestion and standard phenol/chloroform extraction (Hillis et al. 1996). Microsatellite loci for each target species (or closely related species) were available from previously published studies (Table B.1). For each species, microsatellite fragments were amplified as 10 µl reactions containing 1.0 µl DNA (10-50 ng), 1X Colorless GoTaq® Flexi Buffer, 2.5 mM $MgCl_2$ solution, 125 µM dinucleotide triphosphates (dNTPs), 0.4 µM of forward (labeled) and reverse primers, and 0.375 units of GoTaq® DNA Polymerase. Multiplex reactions containing up to three loci were optimized depending on annealing temperature, size range, and fluorescent label for rapid genotyping of individuals. PCR profiles consisted of 90 °C for 3 min initial denaturation followed by 30 cycles of 90 °C for 30

sec, primer-specific annealing temperature (Table B.1) for 30 sec and 72 °C for 45 sec, and ending with a final extension at 72 °C for 15 min. Sample preparation for capillary electrophoresis consisted of 1 µl of PCR product mixed with 10 µl formamide and 0.35 µl of HD400 size standard (ABI) then denatured at 90 °C for five minutes. All samples were run on an automated ABI 3130 DNA sequencer and scored using Genemapper software (ABI). Approximately 10% of samples for each species were reanalyzed and scored to validate allele calls and ensure scoring accuracy.

Spatial Genetic Variation

Tests for departures from Hardy-Weinberg proportions (HWP) within each sample location and a global test for linkage disequilibrium (LD) were performed using GENEPOP (Raymond and Rousset 1995, Rousset 2008). A sequential Bonferroni correction (Rice 1989) was applied to account for inflated Type I error rates associated with multiple comparisons. The program MICROCHECKER (Van Oosterhout et al. 2004) was used to examine the data for null alleles, scoring errors, and large allele dropout which could lead to significant departures from HWP. We evaluated spatial genetic variation for each species using Weir and Cockerham (1984) F-statistics. We estimated overall population differentiation, measured as global F_{ST}, for each species using FSTAT (Goudet 1995). FSTAT implements a bootstrap procedure to test the null hypothesis that the observed F_{ST} value is significantly different from zero. We characterized fine-scale genetic structure between samples for each species by estimating F_{ST} values between all pairs of samples using Arlequin 3.0.1 (Excoffier et al. 2005). Arlequin implements a permutation test for significance of pairwise F_{ST} values. We evaluated the effect of isolation-by-distance (IBD) on genetic structure for each species

by performing Mantel's test on Slatkin's linearized F_{ST} (Rousset 1997) and stream distance between sites. River distances in kilometers were estimated from Google Earth and log transformed to approximate normality for analysis. Mantel tests were performed in R (ver. 2.15.0; R Core Team 2012) using the ecodist package (Goslee & Urban 2007).

Extrinsic influences on genetic structure

We used a landscape genetic approach to evaluate extrinsic factors related to population structuring for each species. For our null hypothesis we expected stream distance to have the strongest influence on linearized F_{ST} (i.e., isolation-by-distance). Alternatively, we hypothesized that certain confluences in the stream network could exert greater influence on genetic differentiation than distance alone. Therefore we created a triangular matrix representing the number of these confluences between pairs of sample locations. Although we considered including difference in elevation between sample locations as an explanatory variable, this was strongly correlated with $\log_{10}$ transformed stream distance (Mantel correlation $r = 0.77$, $P = 0.001$) and was therefore not included.

Distance and tributary architecture matrices estimate the effect of extrinsic factors on gene flow without considering the effect genetic drift may have on pairwise F_{ST}, which can also increase differentiation between populations with low N_e. Pilger et al. (2015) identified low N_e for three focal species in this comparison, suggesting drift in local populations could have large effects on genetic differentiation in these taxa. Therefore, we estimated genetic effective size for each species at each location using the sibship method (N_{eS}) implemented in COLONY (Wang 2009). COLONY uses maximum likelihood to estimate probabilities of full and half siblings of a sample of individuals

taken from a population, from which N_{eS} can be estimated. We constructed pairwise matrices of the harmonic mean of N_{eS} between each pair of sample locations to evaluate the effects of genetic drift on population structuring following Weckworth et al. (2013).

We evaluated the strength of extrinsic (i.e., riverscape architecture) variables (distance, number of confluences, and harmonic mean of N_{eS}) on spatial genetic variation using an information theoretic approach in combination with multiple regression-on-distance matrices (MRM). This approach allowed us to evaluate combinations of explanatory variables while accounting for the non-independence of distance matrices. Initially, seven standard linear models (function *lm*) were fit to linearized F_{ST} representing all combinations of explanatory variables for each species except for *T. cobitis*. Only three models were fit for *T. cobitis* incorporating distance and N_{eS} because the species only occurred in the mainstem Gila River with no intervening confluences. Akaike's information criteria (AIC) was used to select the best candidate models by simultaneously comparing all models. AIC scores were adjusted for small sample size (AIC_C) and Akaike weights (w_i) were calculated using the AICcmodavg package in R (Mazerolle 2015). Candidate models with lowest AIC_C ($\Delta AIC_C < 2.0$) and highest weights ($w_i > 0.10$) were retained for interpretation (Burnham & Anderson 2002) and further evaluated using MRM from the ecodist package (function *MRM*) to identify the contribution of explanatory variables to the overall fit of the model (MRM r^2).

Intrinsic influences on genetic structure

We also used an information theoretic approach with linear regression to evaluate influences of life history and demographic properties on the degree of genetic structuring

among species. We used two genetic summary statistics, global F_{ST} and Mantel's *r* between stream distance and pairwise F_{ST} as dependent variables for each species. Life history attributes (asymptotic body size [mm], length at maturation [mm], fecundity [total number of offspring per breeding season], and egg size [mm diameter]) were available from Olden et al. (2006) (Table 2.1) and used as independent variables in multiple regression. In addition to life history, we suspected that genetic drift acting independently for each taxon could influence levels of differentiation. Here, we hypothesized a negative relation between N_e and global F_{ST} as species with low N_e would be subject to greater drift and thus higher overall differentiation. Individuals were pooled across samples to estimate species N_{eS}. As an alternative approach, we estimated N_{eD} using the linkage disequilibrium method implemented in LDNE (Waples & Do 2008), excluding rare alleles (P_{Crit} = 0.02). Finally, total study distance over which a species occurred (Study-KM; the maximum distance between populations [km]) was included to evaluate the relationship between range size and the degree of genetic structuring. All independent variables, except egg size, were log-transformed to improve approximation to normality of residuals. We tested for collinearity among independent variables using Pearson's correlations (*r*). N_{eS} was highly correlated with Study-KM and N_{eD} ($r > 0.70$) whereas length at maturity was highly correlated with asymptotic body size (max length) and egg size ($r > 0.70$). Both N_{eS} and length at maturity were removed from analyses leaving three life history variables (max length, fecundity, and egg size), N_{eD}, and study distance. Seven candidate models incorporating each variable by itself, all life history variables combined, and all life history variables with study distance held constant, were analyzed

to assess factors contributing to genetic structuring among species. Best models were assessed using the AIC_C and w_i criteria described above and retained for interpretation.

Results

Of the nine species studied, *A. chrysogaster*, *P. clarkii*, and *C. insignis* were the most widespread occurring at 13, 12, and 12 of 16 sample locations, respectively (nine sites had sufficient sample sizes for analysis of *C. insignis*). *Tiaroga cobitis* and *G. nigra* occurred at the fewest sites (four and five sites, respectively, Table B.2). In all but two species, the number of significant deviations from HWP were consistent with the experiment-wise error rate of $\alpha = 0.05$ (Waples 2015) and the application of a Bonferroni correction resulted in low frequency of significant comparisons (< 0.04). The number of significant HWP tests for *M. fulgida* and *T. cobitis* exceeded what would be expected at $\alpha = 0.05$ and each had 15% of tests significant after correction for multiple comparisons. The majority of significant tests were due to heterozygote deficiency, and MICROCHECKER indicated null alleles as the causal factor. All species had at least one significant test for LD but these were not locus or site specific.

All species, except for *A. natalis*, exhibited low but significant genetic structuring with global F_{ST} values ranging from 0.008 (*T. cobitis*) to 0.042 (*R. osculus*; Table 2.1). Five of nine species had a significant Mantel test for the relationship between stream distance and linearized F_{ST} (Figure 2.2). Overall population N_{eS} ranged from 31 (*A. natalis*) to 332 (*A. chrysogaster*) and N_{eD} ranged from 116 (*M. dolomieu*) to 1617 (*C. insignis*; Table 2.1).

Extrinsic influences on genetic structure

Three species, *A. chrysogaster*, *R. osculus*, and *C. insignis* each had four competing models including the full model according to selection criteria ($\Delta AIC_C < 2.0$ and $w_i > 0.10$) (Table 2.2). However, they exhibited contrasting patterns regarding which variables consistently occurred in competing models. Number of confluences occurred in all competing models for *A. chrysogaster* with distance and N_{eS} each present in two competing models. The highest ranked model for *A. chrysogaster* included number of confluences and N_{eS} (MRM $r^2 = 0.22$). Conversely, distance was the strongest predictor for *R. osculus* genetic structure (MRM $r^2 = 0.94$) but including number of confluences and N_{eS}, as in the full model, did not substantially increase explanatory power over distance alone (Table 2.2). Lastly, spatial genetic variation of *C. insignis* was most explained by a negative association with N_{eS} (MRM $r^2 = 0.88$). Explained variation did not increase substantially by including additional variables.

Three species each had a single model with the majority of support. Both *T. cobitis* and *A. natalis* had distance identified as the strongest predictor of differentiation (MRM $r^2 = 0.96$ and 0.43, respectively; Table 2.2). Inclusion of N_{eS} with distance increased explained variation in *P. clarkii* F_{ST} values (MRM $r^2 = 0.73$) over distance alone, because the distance alone model was not well supported. Genetic variation of *M. fulgida* was similarly explained by distance and N_{eS} and had two models with high support.

Two species, *G. nigra* and *M. dolomieu* had three modes were retained each consisting of only variable apiece. Although N_{eS} alone explained approximately 30% of variation of *G. nigra* F_{ST} (MRM $r^2 = 0.31$), *M. dolomieu* was weakly associated with any of the explanatory variables (MRM $r^2 = 0.09$).

Intrinsic influences on genetic structure

There were five competing models for explaining variation in global F_{ST} among species (Table 2.3), yet each model had low adjusted r^2 (-0.14 to 0.08) suggesting none of the variables we included were sufficient in predicting global F_{ST}. The highest ranked model predicted a negative relationship between F_{ST} and egg size (Figure 2.3). Using the same set of factors to predict variation in Mantel's *r* among species, only one high ranking model was identified including only fecundity. Fecundity was negatively associated with Mantel's *r* and explained a substantial amount of variation across species (adj. $r^2 = 0.50$).

Discussion

In this study, we examined varying mechanisms in which intrinsic factors (i.e., life history) interact with extrinsic factors (i.e., riverscape architecture) to produce emergent patterns of neutral genetic variation across nine sympatric fish species. We expected riverscape attributes to generate similar genetic patterns across species, and this expectation was generally met by distance but not number of confluences. Five focal species exhibited strong associations between distance and pairwise F_{ST}, whereas the number of confluences appeared important for only *A. chrysogaster*. Patterns of genetic connectivity varied across species and were related to taxon-specific dispersal and population size. Tests for effects of life history traits on levels of genetic differentiation across species found equivocal results for egg size as a predictor of global F_{ST} across species. However this result contrasts with studies from other stream fish (Turner & Trexler 1998). One reason for this finding could be that within the spatial extent of the

upper Gila River, all focal species generally exhibited a high degree of genetic connectivity as indicated by relatively low F_{ST} values. However, patterns of spatial genetic structure across species, as measured by Mantel's *r*, were strongly associated with fecundity, suggesting that life history traits influence how species interact with the landscape. Below, we discuss our findings in greater detail and provide further life history context to species-specific genetic patterns, but first, we address some limitations of our data.

Implicit in our analyses was the assumption that sample sizes were representative of populations, and thus communities, where sampling occurred. Sample sizes for four species were low ($n < 10$) at some sites and were therefore excluded from landscape genetic analyses to prevent these samples from influencing results. In addition, our sampling design (i.e., collecting members of the same cohort) prevented us from sampling adults at locations where no juvenile individuals occurred. Excluding these putative populations could influence results of spatial genetic variation for these species. However, global estimates of F_{ST} and N_{eD} using all individuals of a species regardless of sample size were similar to estimates that excluded small samples. Furthermore, global N_e estimates correspond to relative abundances of these species from recent and long-term investigations (Propst et al. 2008; Whitney et al. 2014). Despite sample size limitations, patterns of genetic structure across the landscape are consistent with other studies testing the influence of life history and landscape on stream fish population structure (Turner & Trexler 1998; Gomez-Uchida et al. 2009; Fluker et al. 2014).

Two of the more abundant warm-water nonnative species (*A. natalis* and *M. dolomieu*,) were included because they possess divergent life history characteristics from

native fishes that have evolved with the natural flow regime of western streams (Olden et al. 2006). These nonnatives therefore, would increase the scope of life histories not reflected by natives. They are also apex predators in the system and may act as a biotic barrier to gene flow for native species (Pilger et al. 2010). However, both *A. natalis* and *M. dolomieu* have been introduced to the system within the last century and much of their stocking history is unknown. Introductions could have resulted in founder effects (a form of random sampling error caused by few founding individuals) which would immediately reduce genetic diversity. Multiple spatially separated stocking events from genetically differentiated source populations might lead to spurious conclusions regarding natural spatial structuring. We thus compared our finding to those obtained in the native ranges of these species (if available) to aid interpretation.

Extrinsic influences on genetic structure

Distance is often considered a null model n landscape and population genetic analyses because it inherently separates individuals. Specifically, populations that are farther apart in space should be less genetically similar than more proximal populations. Accounting for the spatial autocorrelation of allele frequencies is thus necessary to evaluate the strength of additional mediating factors (Meirmans 2012). For Gila River fishes, distance frequently occurred in competing models of each species but Mantel tests were significant or marginally significant in five of nine species. Of these, *M. fulgida*, *R. osculus*, and *T. cobitis* had the strongest relationship with distance. However, as many researchers have pointed out (Meirmans 2012; Selkoe et al. 2014) and worth noting here, significant relationship with distance does not imply IBD. *Meda fulgida* and *R. osculus* exemplify this problem because although distance explains much of the variation in F_{ST},

further examination of plotted data suggests the relationship is driven by regional structuring. Regional or hierarchical structuring can mimic IBD at large spatial scales, but the strength of that correlation decreases at small, local scales where individuals are essentially panmictic (Meffe & Vrijenhoek 1988; Hughes et al. 2009). Examination of *M. fulgida* and *R. osculus* plots revealed clusters of points, representing either sites closely situated with low pairwise F_{ST} or sites separated by longer distances with high pairwise F_{ST}. At smaller spatial scales (i.e., within a cluster) the relationship between distance and F_{ST} disappears. In contrast, *T. cobitis* exhibited a more incremental increase in F_{ST} with distance indicative of IBD and migration-drift equilibrium (Pilger et al. 2015). A possible reason for differential genetic patterns of *M. fulgida* and *T. cobitis* (both small-bodied minnows) despite being collected from similar locations could be differences in habitat requirements. Habitats occupied by *M. fulgida* tend to be sand and gravel runs that shift temporally, whereas *T. cobitis* occupy cobble riffles that tend to be temporally stable in the Gila River. In addition, greater temporal stability of *T. cobitis* abundance has been reported from long-term surveys (Propst et al. 2008) and could contribute to migration-drift equilibrium and larger N_{eD} relative to *M. fulgida* . *Pantosteus clarkii* genetic variation was associated with distance, but only when accounting for differences in N_{eS}. Lastly, distance outranked all other models for *A. natalis* and explained about 43% of variation of genetic differentiation. Although *A. natalis* exhibited a significant signal of IBD, global and pairwise F_{ST} values were not significantly different from zero. Small sample size and low microsatellite polymorphism for *A. natalis* precluded rejection of panmixia. Ours is the only small-scale landscape genetic study of *A. natalis* or any

congeneric we are aware of, thus we have no reference for comparing genetic patterns of the species in its native range or other introduced ranges.

Within our study area, the Gila River has few impoundments, thus our focus was not to address anthropogenic barriers to gene flow, but instead how natural aspects of the stream network, namely confluences, act as potential barriers to gene flow. Stream confluences are inherent features of riverscape networks that can create sudden geomorphological changes in stream habitat (Benda et al. 2004) and can influence stream fish assemblages (Thornbrugh & Gido 2010). Although we expected the number of confluences to be associated with genetic variation, only one species, *A. chrysogaster*, supported this. However, extrinsic factors explained a small amount of genetic variation for *A. chrysogaster* and for four other species, suggesting genetic drift and additional factors may explain spatial genetic variation of some Gila River fishes.

Until more recently, tests for effects of genetic drift have rarely been conducted on genetic data from natural populations (but see Weckworth et al. (2013)) and therefore remained underappreciated in landscape genetic studies. Effects of drift are usually invoked as a post hoc explanation for unexplained spatial patterns when low N_e is observed. We found that after accounting for distance, the pairwise harmonic mean of N_{eS} to be an important explanatory variable for some but not all species with unexplained spatial patterns. Four species (*G. nigra*, *M. fulgida*, *C. insignis*, and *P. clarkii*) exhibited high variation in local N_{eS} that resulted from one or more locations having relatively low N_{eS}. Strong differentiation can occur between spatially proximal sites with large difference in N_e due to strong genetic drift in the smaller population. These results support previous findings for *G. nigra* and *M. fulgida* that suggest genetic drift to be the

primary force of differentiation in these species (Pilger et al. 2015). However, results for *C. insignis* and *P. clarkii* are somewhat surprising as both are widespread, abundant, and tend to have larger global N_e relative to most other species. We found low N_{eS} for both *C. insignis* and *P. clarkii* in two small tributaries, indicating these smaller streams support fewer breeding individuals and would thus be subject to stronger drift than larger streams leading to higher than expected pairwise F_{ST}.

After accounting for extrinsic factors and genetic drift, three species had substantial unexplained variation in pairwise F_{ST} suggesting alternative intrinsic or extrinsic factors may be important. For example, *M. dolomieu* exhibited significant spatial genetic variation but none of the three predictor variables we tested were correlated with F_{ST}. The pairwise F_{ST} values we observed were similar to those reported for *M. dolomieu* over similar spatial extent in the Laurentian Great Lakes region where the species is native (Stepien et al. 2007). Stepien et al. (2007) observed substantial structure for *M. dolomieu* at small spatial scales without evidence of IBD. They attributed high degree of differentiation to life history traits such as high parental investment (nest building and guarding) and high spawning site fidelity. Five microsatellite loci used in the present study were also used by Stepien et al. (2007) allowing direct comparisons across studies. For these five loci, we observed only slightly fewer alleles per locus (1 to 3 fewer) in the Gila River compared to the entire Laurentian Great Lakes region. In addition heterozygosity values were similar across studies despite our smaller sample sizes. Although we cannot rule out the possibility of founder events influencing our results, it appears that founder events were of low magnitude and the spatial structuring we observed was most likely a result of life history traits of *M. dolomieu*.

Lastly, extirpation and recolonization dynamics can create patterns of genetic variation unrelated to extrinsic factors. Extrinsic factors and harmonic mean of N_{eS} were generally weak predictors of *A. chrysogaster* and *G. nigra* F_{ST} values. Temporal instability of *G. nigra* genetic structure has been observed over a short (two years) time span (Pilger et al. 2015) and it is reported to have the highest probability of extirpation of any focal species in the present study (Chapter 3). High population turnover of *A. chrysogaster* could also obscure genetic patterns. Although widespread and abundant, *A. chrysogaster* can exhibit large demographic fluctuations owing to its "boom-bust" life cycle tendencies (Minckley & Deacon 1968; Minckley & Barber 1971). Founder events in newly formed populations can increase differentiation and genetic drift relative to older populations (Haag et al. 2005; Cosentino et al. 2012). Further characterization of non-equilibrium dynamics of Gila River fishes could shed light on mechanisms influencing genetic differentiation (Chapter 3).

Intrinsic influences on genetic structure

Above we discussed how life history could account for variation in patterns and strength of genetic differentiation across nine sympatric species in the same riverscape. Here we expand on that by explicitly testing whether dispersal related life-history traits influence variation in genetic summary statistics across our focal species. Despite egg size being the highest ranked model for predicting global F_{ST} among species, it explained little variation (adjusted $R^2 = 0.08$). These results contrast with those of Turner and Trexler (1998) who found clutch size, female body size, and egg size were strongly associated with differing levels of gene flow among 15 darter species (family Percidae). Reasons for these contrasting results are various but could involve 1) scale-dependency

of the relationship between dispersal and F_{ST} (Phillipsen et al. 2015), 2) sample size issues, and/or 3) issues related to F_{ST} as a summary statistic. The spatial extent of the Gila River is orders of magnitude smaller than in studies that have found significant relationships between differentiation and life-history traits (e.g., three geographic regions in Turner and Trexler (1998), Hawaiian Archipelago in Selkoe et al. (2014)). For example, three of the species in this study were found to have differing levels of divergence (θ [$\theta \cong F_{ST}$] from allozymes: *A. chrysogaster* < *M. fulgida* < *T. cobitis*) across their entire range in New Mexico and Arizona that matched their presumed dispersal capabilities (Tibbets & Dowling 1996). Within the upper Gila River catchment, these species exhibited an almost opposite pattern (*T. cobitis* < *A. chrysogaster* < *M. fulgida*) based on microsatellite markers. In marine systems, results have been equivocal on the influence of life-history traits on genetic connectivity (e.g., Galarza et al. 2009; Riginos et al. 2011; Selkoe et al. 2014). Lack of statistical significance with life history traits could be due to low sample size (n = 9 species) or strong covariance between body size and other life history traits. Rigninos et al. (2011) also noted substantial amounts of unexplained variation in models predicting F_{ST} for over a hundred species. This, they explain, might be a reason for insignificant results in studies with substantially fewer species. Lastly, we have to consider the metric F_{ST}. Although we equate dispersal with gene flow, F_{ST} does not provide an accurate metric of gene flow when assumptions of the island model are violated (Whitlock & McCauley 1999), as is likely the case in the upper Gila River. Furthermore, F_{ST} only measures degree of allelic differentiation but does not address how variation is distributed across space. More meaning may be gleaned from summary statistics incorporating distance such as Mantel's *r*.

In linearized systems such as streams, Mantel's *r* provides a summary of spatial distribution of genetic variation. We found fecundity to be negatively associated with Mantel's *r* suggesting that, in general, species with low fecundity showed increasing correlation with distance. Selkoe et al. (2014) found habitat depth range, rather than dispersal factors, were strong predictors of Mantel's *r* for a number of coral species. Meirmans et al. (2011) also used Mantel's *r* as a summary statistic but found spatial genetic variation in alpine plants was explained by soil type which was strongly influenced by glaciation history and not dispersal factors. These studies highlight the difficulty using descriptive summary statistics because of the complicated relationship between historical factors and ongoing gene flow (Meirmans 2012). Two of our species clearly show hierarchical structuring (*M. fulgida* and *R. osculus*) accounting for their strong associations with distance. In future analyses, statistical approaches that account for spatial-autocorrelation of allele frequencies and other factors could be used to separate the effects of spatial processes from life history and its influence on genetic structure (Meirmans 2012; Manel & Holderegger 2013). While it is important to note that different mechanisms are likely responsible for the spatial genetic variation, the comparative approach allowed us to see an emergent trend. For species in linear habitats, distance tends to be a weak isolating mechanism for highly fecund species but a strong isolating mechanism for low fecundity species.

Conclusion

Understanding the ecological mechanisms responsible for genetic differentiation of subdivided populations is a central question in the fields of evolutionary ecology and conservation genetics because 1) over long time scales, divergence of genetic lineages is

a precursor to speciation (Coyne & Orr 2004) and 2) over short time scales, species evolutionary response (i.e., genetic diversity and structuring) to metapopulation processes and conservation threats are mediated by patterns of genetic connectivity (Whitlock & McCauley 1990; Whitlock & Barton 1997). For example, Gila River fishes generally exhibited a high degree of genetic connectivity as indicated by relatively low F_{ST}. Maintaining functional connectivity will be important for limiting further reduction of genetic diversity for all species in this system. However, because distance has a stronger isolating effect on low fecundity species (*A. chrysogaster, M. fulgida. R. osculus* and *T. cobitis*), they are most likely to experience rapid declines in genetic diversity if connectivity is reduced. Alternatively, for the more fecund native species (*G. nigra, C. insignis*, and *P. clarkii*), factors associated with reducing local N_e, such as negative interactions with nonnative species or disturbance, are most likely to increase the overall effect of drift, thereby reducing metapopulation genetic effective size. Lastly, the species-specific responses observed here further stress the importance for ecological context when interpreting genetic data.

Literature Cited

Apodaca JJ, Trexler JC, Jue NK, Schrader M, Travis J (2013) Large-scale natural disturbance alters genetic population structure of the sailfin molly, *Poecilia latipinna*. American Naturalist, 181, 254-263.

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, 63, 62-76.

Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University Press.

Banks SC, Cary GJ, Smith AL, Davies ID, Driscoll DA, Gill AM, Lindenmayer DB, Peakall R (2013) How does ecological disturbance influence genetic diversity? Trends in Ecology & Evolution, 28, 670-679.

Benda L, Poff NL, Miller D, Dunne T, Reeves G, Pess G, Pollock M (2004) The network dynamics hypothesis: how channel networks structure riverine habitats. BioScience, 54, 413-427.

Blanchet S, Rey O, Etienne R, Lek S, Loot G (2010) Species-specific responses to landscape fragmentation: implications for management strategies. Evolutionary Applications, 3, 291-304.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. Quarterly Review of Biology, 74, 21-45.

Burney CW, Brumfield RT (2009) Ecology predicts levels of genetic differentiation in Neotropical birds. American Naturalist, 174, 358-368.

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media, New York, New York.

Cosentino BJ, Phillips CA, Schooley RL, Lowe WH, Douglas MR (2012) Linking extinction-colonization dynamics to genetic structure in a salamander metapopulation. Proceedings of the Royal Society B-Biological Sciences, 279, 1575-1582.

Coyne JA, Orr HA (2004) Speciation. W.H. Freeman. pp. 545.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1, 47-50.

Faurby S, Barber PH (2012) Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Molecular Ecology, 21, 3419-3432.

Fluker BL, Kuhajda BR, Harris PM (2014) The influence of life-history strategy on genetic differentiation and lineage divergence in darters (Percidae: Etheostomatinae). Evolution, 68, 3199-3216.

Galarza JA, Carreras-Carbonell J, Macpherson E, Pascual M, Roques S, Turner GF, Rico C (2009) The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. Proceedings of the National Academy of Sciences of the United States of America, 106, 1473-1478.

Givnish TJ (2010) Ecology of plant speciation. Taxon, 59, 1326-1366.

Gomez-Uchida D, Knight TW, Ruzzante DE (2009) Interaction of landscape and life history attributes on genetic diversity, neutral divergence and gene flow in a pristine community of salmonids. Molecular Ecology, 18, 4854-4869.

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software, 22, 1-19.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity, 86, 485-486.

Haag CR, Riek M, Hottinger JW, Pajunen VI, Ebert D (2005) Genetic diversity and genetic differentiation in Daphnia metapopulations with subpopulations of known age. Genetics, 170, 1809-1820.

Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. Bulletin of Marine Science, 87, 971-1002.

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: Sequencing and cloning. Molecular systematics, 2nd edn. Sinauer Associates, Sunderland. p.^pp.

Hughes JM, Huey JA, Schmidt DJ (2013) Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? Freshwater Biology, 58, 951-966.

Hughes JM, Schmidt DJ, Finn DS (2009) Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. Bioscience, 59, 573-583.

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution, 38, 1371-1383.

Koizumi I, Yamamoto S, Maekawa K (2006) Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. Molecular Ecology, 15, 3175-3189.

Manel S, Holderegger R (2013) Ten years of landscape genetics. Trends in Ecology & Evolution, 28, 614-621.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution, 18, 189-197.

Manier MK, Arnold SJ (2006) Ecological correlates of population genetic structure: a comparative approach using a vertebrate metacommunity. Proceedings of the Royal Society B-Biological Sciences, 273, 3001-3009.

Mazerolle MJ (2015) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 20-3.

Meffe GK, Vrijenhoek RC (1988) Conservation genetics in the management of desert fishes. Conservation Biology, 2, 157-169.

Meirmans PG (2012) The trouble with isolation by distance. Molecular Ecology, 21, 2839-2846.

Meirmans PG, Goudet J, Gaggiotti OE, IntraBioDiv C (2011) Ecology and life history affect different aspects of the population structure of 27 high-alpine plants. Molecular Ecology, 20, 3144-3155.

Minckley WL, Barber WE (1971) Some aspects of biology of the longfin dace, a cyprinid fish characteristic of streams in the Sonoran Desert. The Southwestern Naturalist, 15, 459-464.

Minckley WL, Deacon JE (1968) Southwestern fishes and the enigma of "endangered species". Science, 159, 1424-&.

Morrissey MB, de Kerckhove DT (2009) The maintenance of genetic variation due to asymmetric gene flow in dendritic metapopulations. American Naturalist, 174, 875-889.

Olden JD, Poff NL, Bestgen KR (2006) Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. Ecological Monographs, 76, 25-40.

Phillipsen IC, Kirk EH, Bogan MT, Mims MC, Olden JD, Lytle DA (2015) Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. Molecular Ecology, 24, 54-69.

Pilger TJ, Gido KB, Propst DL (2010) Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: implications for native fish conservation. Ecology of Freshwater Fish, 19, 300-321.

Pilger TJ, Gido KB, Propst DL, Whitney JE, Turner TF (2015) Comparative conservation genetics of protected endemic fishes in an arid-land riverscape. Conservation Genetics, 1-14.

Propst DL, Gido KB, Stefferud JA (2008) Natural flow regimes, nonnative fishes, and native fish persistence in arid-land river systems. Ecological Applications, 18, 1236-1252.

R Core Team (2012) R: A language and environment for statistical computing. Vienna, Austria.

Rice WR (1989) Analyzing tables of statistical tests. Evolution, 43, 223-225.

Riginos C, Douglas KE, Jin Y, Shanahan DF, Treml EA (2011) Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography, 34, 566-575.

Rodriguez MA (2002) Restricted movement in stream fish: The paradigm is incomplete, not lost. Ecology, 83, 1-13.

Rodriguez MA (2010) A modeling framework for assessing long-distance dispersal and loss of connectivity in stream fish. Pages 263-279 *in* Gido KB, Jackson DA, editors. Community ecology of stream fishes: concepts, approaches, and techniques. American Fisheries Society Symposium 73, Bethesda, Maryland..

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics, 145, 1219-1228.

Selkoe KA, Gaggiotti OE, ToBo Laboratory, Bowen BW, Toonen RJ (2014) Emergent patterns of population genetic structure for a coral reef community. Molecular Ecology, 23, 3064-3079.

Skalski GT, Gilliam JF (2000) Modeling diffusive spread in a heterogeneous population: A movement study with stream fish. Ecology, 81, 1685-1700.

Skalski GT, Gilliam JF (2003) A diffusion-based theory of organism dispersal in heterogeneous populations. American Naturalist, 161, 441-458.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, 236, 787-792.

Stepien CA, Murphy DJ, Strange RM (2007) Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian

Great Lakes and beyond: an interplay of behaviour and geography. Molecular Ecology, 16, 1605-1624.

Thornbrugh DJ, Gido KB (2010) Influence of spatial positioning within stream networks on fish assemblage structure in the Kansas River basin, USA. Canadian Journal of Fisheries and Aquatic Sciences, 67, 143-156.

Tibbets CA, Dowling TE (1996) Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). Evolution, 50, 1280-1292.

Turner TF, Trexler JC (1998) Ecological and historical associations of gene flow in darters (Teleostei: Percidae). Evolution, 52, 1781-1801.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4, 535-538.

Wang JL (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Molecular Ecology, 18, 2148-2164.

Waples RS (2015) Testing for Hardy–Weinberg Proportions: Have We Lost the Plot? Journal of Heredity, 106, 1-19.

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources, 8, 753-756.

Weckworth BV, Musiani M, DeCesare NJ, McDevitt AD, Hebblewhite M, Mariani S (2013) Preferred habitat and effective population size drive landscape genetic patterns in an endangered species. Proceedings of the Royal Society B-Biological Sciences, 280, 20131756; DOI: 10.1098/rspb.2013.1756..

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358-1370.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. Genetics, 146, 427-441.

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, 44, 1717-1724.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity, 82, 117-125.

Whitney JE, Gido KB, Propst DL (2014) Factors associated with the success of native and nonnative species in an unfragmented arid-land riverscape. Canadian Journal of Fisheries and Aquatic Sciences, 71, 1134-1145.

Wright S (1931) Evolution in Mendelian populations. Genetics, 16, 97-159.

Tables and Figures

Table 2.1 Genetic characteristics and selected life history traits of nine native and nonnative Gila River fishes sampled in 2010. Maximum body length (L_{Max}), length at maturation (L_{Mat}), fecundity and egg size were obtained from Olden et al. (2006). Genetic summary stats of *Gila nigra*, *Meda fulgida*, and *Tiaroga cobitis* previously reported in Pilger et al. (2015).

Species	F_{ST} (95% CI)	Mantel *r*	N_{eS} (95% CI)	N_{eD} (95% CI)	L_{Max} [mm]	L_{Mat} [mm]	Fecundity (log_{10})	Egg size [mm]	Study KM
Agosia chrysogaster	0.016 (0.012-0.020)	0.18	332 (275-383)	1217 (595-33,322)	100	55	3	1.9	194
Gila nigra	0.027 (0.014-0.041)	-0.24	67 (47-97)	86 (62-131)	405	100	4.4	1.4	48
Meda fulgida	0.021 (0.015-0.026)	0.88*	167 (128-220)	325 (244-470)	91	52	3.1	1.7	140
Rhinichthys osculus	0.042 (0.027-0.062)	0.97**	183 (144-227)	143 (99-224)	110	66	2.7	1.5	208
Tiaroga cobitis	0.008 (0.005-0.011)	0.98**	156 (116-211)	602 (292-20,719)	65	45	2.8	1.3	129
Pantosteus clarkii	0.013 (0.009-0.016)	0.59**	285 (239-346)	496 (331-907)	330	120	4	3.2	167

Species	F_{ST} (95% CI)	Mantel *r*	N_{eS} (95% CI)	N_{eD} (95% CI)	L_{Max} [mm]	L_{Mat} [mm]	Fecundity (log_{10})	Egg size [mm]	Study KM
Catostomus insignis	0.014 (0.011-0.019)	-0.21	190 (152-234)	1617 (605-inf)	800	300	4.1	3.3	167
Ameiurus natalis[1]	0.007 (-0.004-0.024)	0.66**	31 (21-52)	121 (44-2649)	470	233	3.6	3	137
Micropterus dolomieu[1]	0.036 (0.024-0.048)	0.30	46 (31-68)	116 (58-330)	690	205	4.1	2.3	137

* significant at $P < 0.1$, ** significant at $P < 0.05$, [1] Nonnative species

Table 2.2 Multiple regression on distance matrices (MRM) for explaining spatial genetic variation of Gila River fishes sampled in 2010. Models were ranked using AICc and only models with ΔAICc < 2.0 and AICc w_i > 0.10 are reported. Number of parameters in the model (K) includes intercept and error term. Coefficients are for each explanatory variable included in the model with standard error (S.E.). MRM r^2 is the amount of variation explained by the model using MRM analysis.

Models	K	AICc	ΔAICc	w_i	Cum. w_i	Coefficients (S.E.)			MRM r^2
						Distance	Confluence	N_{eS}	
A. chrysogaster									
Confluence + N_{eS}	4	-487.12	0.00	0.37	0.37		8.0E-03 (1.9E-03)	4.3E-04 (2.6E-04)	0.223
Confluence	3	-486.61	0.51	0.29	0.65		5.9E-03 (1.4E-03)		0.195
Distance + Confluence + N_{eS}	5	-485.85	1.27	0.19	0.85	2.3E-05 (2.3E-05)	7.7E-03 (1.9E-03)	4.3E-04 (2.6E-04)	0.233
Distance + Confluence	4	-485.36	1.76	0.15	1.00	2.28E-05 (2.35E-05)	5.59E-03 (1.41E-03)		0.205
M. fulgida									
Distance	3	-95.67	0.00	0.54	0.54	2.75E-04 (4.13E-05)			0.773
Distance + N_{eS}	4	-94.65	1.03	0.32	0.86	2.78E-04 (3.92E-05)		-2.24E-04 (1.43E-04)	0.811
R. osculus									

Models	K	AICc	ΔAICc	w_i	Cum. w_i	Coefficients (S.E.)			MRM r^2
						Distance	Confluence	N_{eS}	
Distance	3	-153.78	0.00	0.38	0.38	7.80E-04 (3.79E-05)			0.942
Distance + Confluence	4	-152.75	1.03	0.22	0.60	7.93E-04 (3.90E-05)	-4.52E-03 (3.61E-03)		0.946
Distance + Confluence + N_{eS}	5	-152.73	1.05	0.22	0.82	7.58E-04 (4.34E-05)	-6.53E-03 (3.70E-03)	-1.04E-03 (6.33E-04)	0.951
Distance + N_{eS}	4	-152.30	1.48	0.18	1.00	7.54E-04 (4.51E-05)		-6.66E-04 (6.22E-04)	0.945
T. cobitis									
Distance	3	-48.22	0.00	1.00	1.00	1.15E-04 (1.21E-05)			0.958
G. nigra									
N_{eS}	3	-23.44	0.00	0.44	0.44			-9.48E-04 (7.10E-04)	0.308
Confluence	3	-23.19	0.25	0.39	0.83		-1.03E-02 (8.29E-03)		0.279
Distance	3	-21.59	1.85	0.17	1.00	-1.21E-04 (2.40E-04)			0.059

Models	K	AICc	ΔAICc	w_i	Cum. w_i	Coefficients (S.E.)			MRM r^2
						Distance	Confluence	N_{eS}	
C. insignis									
N_{eS}	3	-263.51	0.00	0.33	0.33			-1.11E-03 (7.15E-05)	0.876
Confluence + NeS	4	-263.41	0.10	0.31	0.64		2.45E-03 (1.61E-03)	-1.06E-03 (7.64E-05)	0.884
Distance + Confluence + N_{eS}	5	-262.68	0.84	0.21	0.85	3.03E-05 (2.26E-05)	2.93E-03 (1.63E-03)	-1.08E-03 (7.68E-05)	0.890
Distance + N_{eS}	4	-261.94	1.57	0.15	1.00	2.16E-05 (2.28E-05)		-1.13E-03 (7.45E-05)	0.879
P. clarkii									
Distance + N_{eS}	4	-501.05	0.00	0.75	0.75	1.18E-04 (1.36E-05)		-5.74E-04 (6.19E-05)	0.725
A. natalis									
Distance	3	-72.41	0.00	0.70	0.70	1.96E-04 (7.86E-05)			0.436
M. dolomieu									
Distance	3	-72.58	0.00	0.39	0.39	1.34E-04 (1.19E-04)			0.090

Models	K	AICc	ΔAICc	w_i	Cum. w_i	Coefficients (S.E.)			MRM r^2
						Distance	Confluence	N_{eS}	
N_{eS}	3	-71.48	1.10	0.23	0.62			3.12E-03 (6.00E-03)	0.020
Confluence	3	-71.46	1.12	0.22	0.84		-3.95E-03 (7.92E-03)		0.019

Table 2.3 Linear models ranked by AIC_C that predict global F_{ST} and Mantel *r* for Gila River fishes. Only models with $\Delta AIC_C < 2.0$ and Akaike weights (w_i) > 0.10 were retained for interpretation. Values of ΔAIC_C represent the change in AIC_C relative to the highest ranking model and K is the number of parameters in the model (including the intercept and error term). Variable coefficients (β) and standard errors (β SE) are indicated along with R^2 and adjusted R^2.

	Model Variables	K	AICc	ΔAICc	w_i	Cum. w_i	β	β SE	R^2	Adj. R^2
F_{ST}	Egg size	3	-46.92	0.00	0.32	0.32	-0.0063	0.0049	0.19	0.08
	N_{eD}	3	-46.86	0.06	0.31	0.63	-0.0044	0.0035	0.19	0.07
	Fecundity	3	-45.03	1.89	0.12	0.75	-0.0009	0.0068	2.65E-03	-0.14
	Study km	3	-45.02	1.90	0.12	0.88	-0.0009	0.0102	1.19E-03	-0.14
	L_{Max}	3	-45.01	1.91	0.12	1.00	-0.0003	0.0045	4.49E-04	-0.14
Mantel *r*	Fecundity	3	14.36	0.00	0.70	0.70	-0.5550	0.1835	0.57	0.50

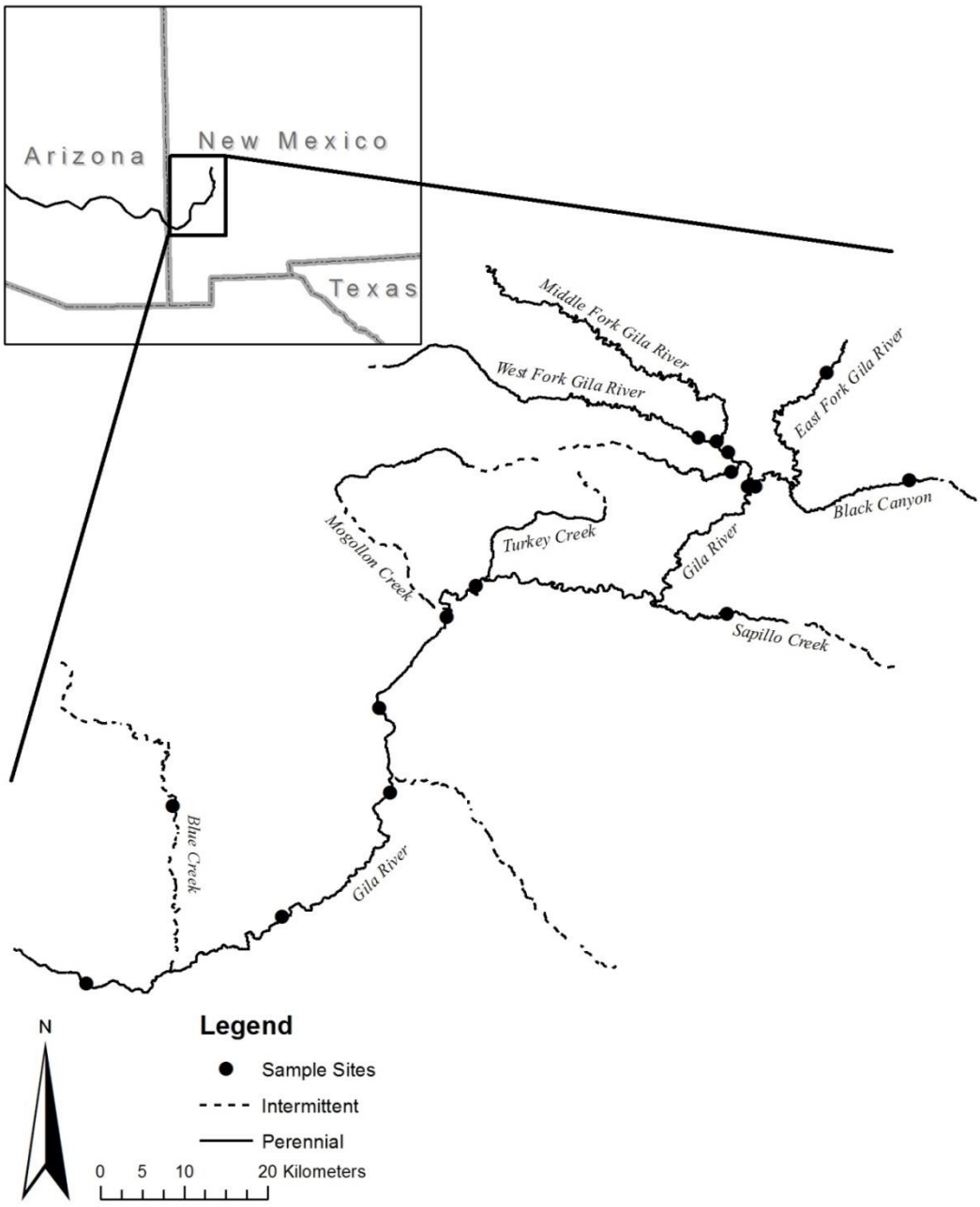

Figure 2.1 Sample locations in the upper Gila River basin, New Mexico, USA.

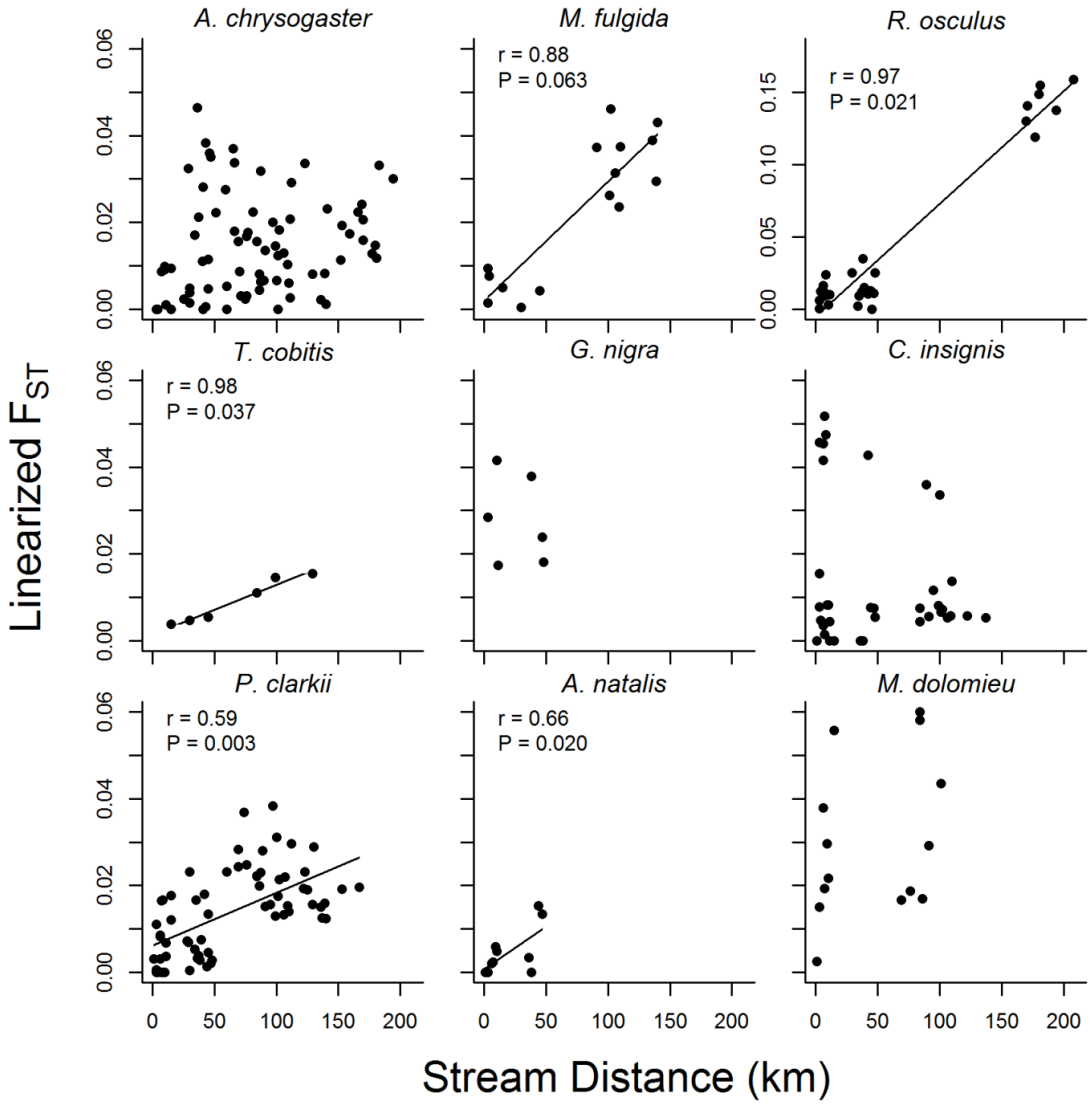

Figure 2.2 Scatter plots depicting the relationship between genetic distance (linearized F_{ST}) and stream distance for nine Gila River fishes sampled in 2010. Least-squares regression lines are shown for those species with significant Mantel tests. Note the difference in scale on the y-axis for *R. osculus*, all x-axes are identical.

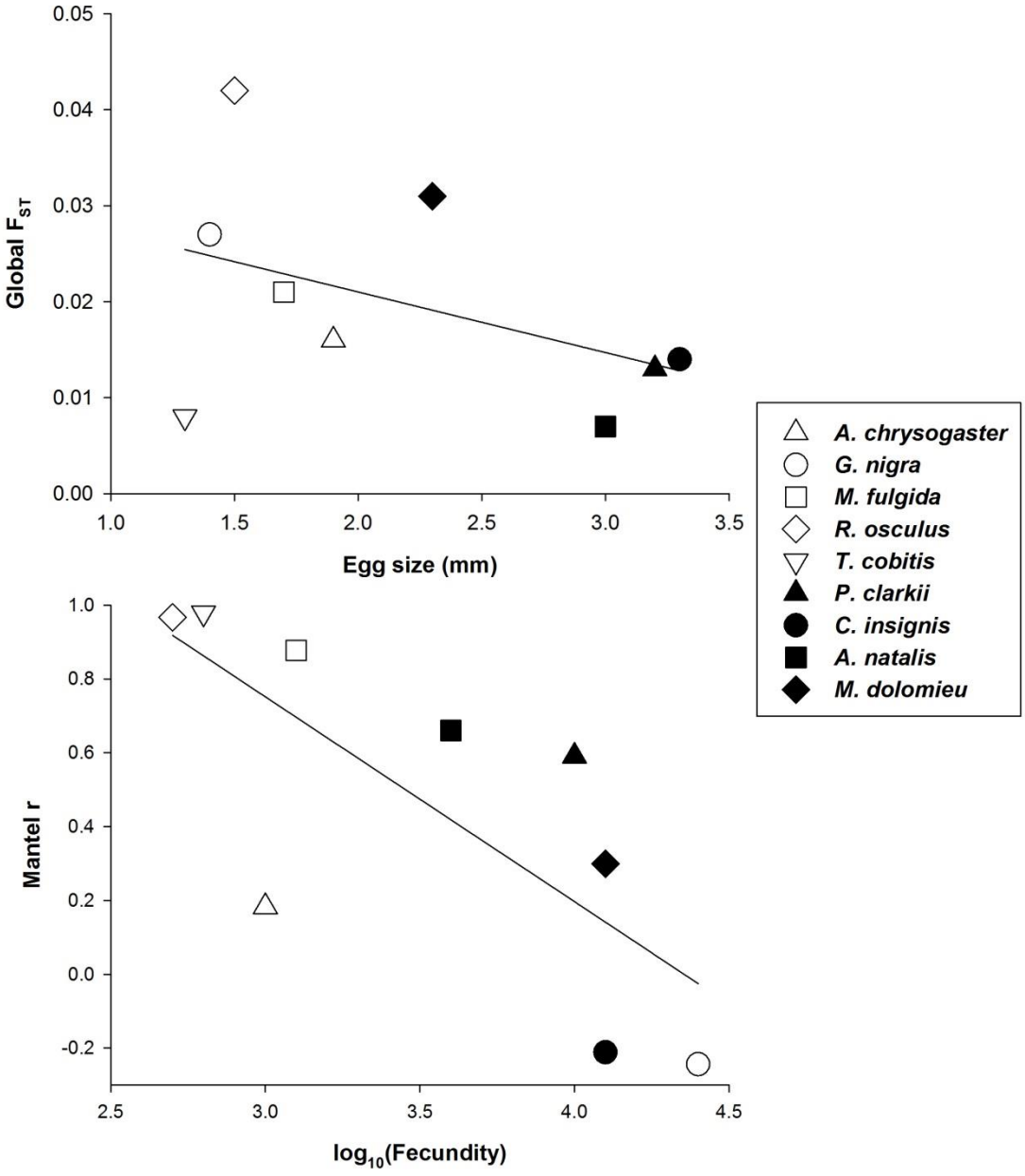

Figure 2.3 . Bivariate relationships between life history and genetic variation statistics across nine Gila River fishes sampled in 2010. Only the highest ranked models for global F_{ST} (top panel) and Mantel *r* (bottom panel) are presented (full results presented in Table 2.3).

Chapter 3

METACOMMUNITY DYNAMICS AFFECT GENETIC DIVERSITY

Abstract

There is long theoretical history behind metapopulations and how colonization and extirpation processes should influence both genetic variation and diversity. Analytical models have become incrementally more complex beginning with Wright's simplistic infinite islands model to incorporating elements of Levins's metapopulation model and environmental stochasticity. These analyses revealed more complicated yet predictable relationships among population subdivision, genetic variation, and genetic effective size (N_e) in metapopulations. Yet, there is scant empirical evidence testing the relationship between metapopulation processes and genetic diversity. We used a combination of genetic and ecological data to 1) evaluate the influence of nonequilibrium processes of extirpation and recolonization on total genetic effective size of the metapopulation (meta-N_e), and 2) characterize the role of life history and other intrinsic differences on shaping species' evolutionary potential through their effects on meta-N_e. Genetic and ecological data were incorporated into meta-N_e using four separate models that account for different demographic processes and spatial configurations. Temporal instability in the form of extirpations reduced meta-N_e (regardless of estimation method) across species with greater local extirpation rates. However, greater abundance of some species could counter the effect of extirpation. Our results support the conclusion that trade-offs related to life history strategies could lead to different consequences for maintaining genetic diversity of species. In addition, we evaluated different meta-N_e methods and found they may be

informative for identifying demographic processes affecting meta-N_e, such as asymmetric gene flow and variance among subpopulation N_e. However, models were sensitive to different assumptions, and thus await further investigation. By combining demographic and genetic data, we show that summary statistics employed in conservation studies capture essential demographic features of metapopulations with respect to their persistence on the landscape.

Introduction

Understanding demographic and ecological mechanisms affecting species' evolutionary trajectory by increasing or decreasing genetic diversity is a critical component of evolutionary ecology and biodiversity conservation. The effective size of a population (N_e) is an evolutionarily important metric describing the rate at which neutral genetic diversity is lost in populations (Wright 1931). Genetic variation is maintained and lost via four fundamental processes: gene flow, genetic drift, mutation and selection, all of which are directly modulated by N_e. For example, selection, a primarily deterministic process, can be a strong force when N_e is large, keeping deleterious mutations at low frequency and even overcoming gene flow. However, when N_e becomes small, the stochastic process of genetic drift becomes the dominant force responsible for reduction of genetic diversity and fixation of deleterious mutations. At sufficiently small N_e, drift can overpower gene flow, the primary force of increasing genetic diversity in populations (Frankham et al. 2009). Whereas N_e is a seemingly straight-forward concept, estimating N_e in natural populations has unrealistically simple assumptions and much effort has been invested in understanding how additional complexity in biological systems affects N_e (Wang & Caballero 1999; Ray 2001; Whitlock 2004).

A major challenge in estimating N_e is that species in natural settings rarely behave in the idealized manner in which theoretical models derive estimates of N_e. For example, most species do not exist as a single, large, panmictic population; instead they inhabit heterogeneous landscapes leading to spatially structured populations. Population subdivision leads to differences in allelic frequencies among different populations, the effects of which are counteracted by gene flow. Whereas N_e of a single, panmictic population is dependent solely on population size, for subdivided populations N_e relies on a balance of genetic drift within populations and gene flow among populations (Wright 1943). Specifically, when species are divided into subpopulations, two processes occur, namely 1) as the rate of gene flow decreases between populations, differentiation of allelic frequencies between populations increases (measured as F_{ST}), and 2) increasing population subdivision reduces the probability of a given allele going to fixation, thereby increasing N_e over the entire metapopulation (herein referred to as meta-N_e) relative to a single panmictic population of the same size. However, increasing meta-N_e will only occur under the strictest of assumptions (Wright 1943). Subsequent theoretical investigations of meta-N_e studied the effects of relaxing basic assumptions of Wright's model (i.e., among population variance, spatial configuration, asymmetrical gene flow, extirpation/recolonization dynamics, see reviews by Wang and Caballero (1999) and Whitlock (2004)). Conclusions from this theoretical work indicate that meta-N_e will only increase, relative to the sum of all subpopulation N_e values (ΣN_es, herein, N_es refers to the plural form of N_e), under conditions that constrain variance in reproductive success across subpopulations (Whitlock 2004). However, factors that enhance variance in reproductive output among populations such as variance in subpopulation N_es,

asymmetry in subpopulation contribution, or extirpation/recolonization dynamics will decrease meta-N_e relative to ΣN_es (Whitlock and Barton 1997, Nunney 1999). Reduction of meta-N_e relative to ΣN_es is analogous to variance in reproductive success among individuals in single, interbreeding population reducing the effective size to census size ratio.

Although theory predicts a general decrease in meta-N_e for natural metapopulations, few empirical studies have explicitly tested these predictions. Population subdivision was reported to increase N_e in prairie dogs (Sugg et al. 1996), but see Nunney (1999)). However, several studies on salmonid fishes provide support for population subdivision decreasing meta- N_e relative to ΣN_es (Fraser et al. 2007; Kuparinen et al. 2010; Palstra & Ruzzante 2011). Asymmetric gene flow in idealized spatial configurations (i.e., island model, stepping stone) is expected to decrease meta-N_e, but for species with hierarchical genetic structuring in dendritic networks, asymmetric gene flow maintains high levels of genetic diversity leading to high meta-N_e (Morrissey & de Kerckhove 2009). Yet, studies from salmonid fishes reported asymmetric gene flow decreased meta-N_e relative to ΣN_es (Kuparinen et al. 2010; Gomez-Uchida et al. 2013). Lastly, temporal instability in the form of frequent extirpations and recolonizations reduced meta-N_e in a metapopulation of mycophagous beetles (Ingvarsson et al. 1997). Elsewhere, metapopulations of ephemeral midge species experiencing repeated local extirpations were reported to have higher regional genetic diversity (and presumably increased meta-N_e) than permanent species (Berendonk et al. 2009). With the exception of Berendonk et al. (2009) whose study included six species, previous research has focused on three or fewer species. These few and contrasting

studies highlight the complexities involving life history, metapopulation dynamics, and meta-N_e and the need for more empirical studies across multiple species with varying life histories.

In this study we focus on nine of the most numerically abundant species comprising the warm-water fish community of the Gila River, New Mexico, USA. Our previous research has identified differential genetic responses to landscape features related to divergent life-history strategies across focal species, but some species exhibited patterns of genetic structure unrelated to landscape features which might be due to nonequilibrium dynamics (See chapter 2). Here, we combine previous research on factors that shape species-specific genetic patterns with contemporary abundance data and presence/absence data from nearly 30 years of record to 1) evaluate the influence of nonequilibrium processes of extirpation and recolonization on N_e and meta-N_e, and 2) characterize the role of life history and other intrinsic differences on shaping species' evolutionary potential through their effects on meta-N_e. First, we test the hypothesis from theoretical predictions that increasing temporal instability across species, as measured by probability of extirpation, is inversely related to species' genetic effective size. Second we compared the behavior of different meta-N_e models to evaluate their relevance for identifying factors important for reduced meta-N_e relative to ΣN_es if assumptions related to different models are violated, such as asymmetric gene flow, temporal instability, and variation in local population size. Lastly, we report on how ecology affects the evolutionary trajectory of species by demonstrating that species-specific deviations from theoretical predictions are related to species' life histories. We

discuss our findings within the context of biodiversity conservation and factors related to maintaining genetic variation within species in the Gila River system.

Methods

Study system and ecological data

We evaluated the influence of nonequilibrium dynamics on meta-N_e with data from nine, distantly related species co-occurring in the upper Gila River basin in southwestern New Mexico, USA (Figure 2.1). Within our study area, the Gila River has few anthropogenic alterations and only one major impoundment at the downstream end of the study area. Like many other lotic systems in arid regions, the Gila River is a highly variable system characterized by high spring runoff (March to April) and flashy spates occurring during the annual monsoon season (July to September). Furthermore, flow intermittency in tributaries and mainstem valley segments (often going dry during summer months) likely results in local extirpations. The variable nature of the Gila River makes it an ideal system to evaluate the effects of population turnover from extirpations on patterns of genetic variation across species. Metapopulation probabilities (occupancy, extirpation, colonization) were estimated for each species as described in Whitney et al. (2015) using a long-term dataset (27 years; see Propst et al. (2008) for sampling details) from locations that coincide with four sample locations in the current study. Although metapopulation probabilities describe several demographic aspects, we were only interested in probability of extirpation (P_e: number of extirpation events/number of extirpation opportunities) for quantifying temporal instability (Table 3.1). In addition to long-term P_e, we expected contemporary abundances to influence species' N_e; therefore

estimates of mean density (m^{-2}) were measured bi-annually from 2008 to 2010 at six locations coinciding with this study (see Whitney et al. (2015) "Pre-fire sites").

Genetic data

Focal species represent four families of Teleost fishes in which previous landscape genetic analyses identified quantitative differences in spatial genetic structure and contemporary effective size related to landscape (i.e., distance) and life history (i.e., fecundity) (Chapter 2). Sample sizes within species varied across locations; therefore we set a minimum sample size of 10 individuals per location for estimating subpopulation N_es. This resulted in exclusion of 1 location each for *Ameiurus natalis* and *Gila nigra,* 3 locations for *Catostomus insignis*, and 4 locations for *Micropterus dolomieu*. Contemporary N_es of subpopulations (at each sample location) were estimated for each species using the linkage disequilibrium (LD) method implemented in LDNE (Waples & Do 2008). Because of our relatively small sample sizes at each location ($10 \leq n \leq 30$ individuals) we set the cut-off (P_{crit}) to exclude low frequency alleles at $P_{crit} = 1/2S$, where S is the sample size (Waples & Do 2010). In some instances, adjusting P_{crit} still resulted in negative or overly large estimates with infinite confidence intervals. In these cases, the lower 95% CI was used as an approximate N_e value (Waples & Do 2010).

Models of meta-N_e require population level genetic parameters, including global differentiation (F_{ST}), number of subpopulations (n) and contemporary migration rates (m; Table 3.1). Global F_{ST} values for each species were taken from Chapter 2. For n, we used the number of sample locations for which we estimated subpopulation N_es. Contemporary migration (m) was estimated for each species using a Bayesian inference

approach implemented in BAYESASS 3.0.3 (Wilson & Rannala 2003). For each species, three to five short runs were used to tune parameter acceptance rates followed by five independent long runs consisting of 10^7 iterations, of which the first 10^6 were discarded as burn-in, and a sample size of 100 iterations. Convergence of MCMC chains was visually assessed using the program Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). BAYESASS provides unidirectional migration estimates between population pairs which were first averaged (i.e., bidirectional *m* between sites) to estimate overall mean *m* for each species.

Effective metapopulation size (meta-N_e)

A number of methods for estimating meta-N_e have been derived from models that relax various assumptions of Wright's (1943) island model (Wang & Caballero 1999; Table 3.2), yet recent empirical studies on salmonid fishes in stream networks found classic spatial models (e.g., stepping stone (Maruyama 1970) and linear neighborhood (Wright 1946; Maruyama 1971)) to be rather uninformative for applications with empirical data (Palstra & Ruzzante 2011; Gomez-Uchida et al. 2013). Our primary interest was in testing the estimate of Whitlock and Barton (1997) that explicitly accounts for extirpation/recolonization effects on meta-N_e. Neither of the recent studies tested the Whitlock and Barton (1997) estimate; however, both noted an eigenvalue method developed by Tufto and Hindar (2003) to vary across species according to demographic processes, particularly asymmetric dispersal.

In this study we estimated meta-N_e using four methods. First, we estimated ΣN_es, the sum of local effective sizes across all sample locations, to serve as a null model with

which to compare meta-N_e estimates based on different sets of assumptions and spatial migration models (see Table 3.2). The second estimate of metapopulation effective size was estimated by pooling all individuals across sites and calculated using LDNE ($N_{e\ pool}$). Others have noted strong downward bias of $N_{e\ pool}$ introduced by differentiation among populations (Palstra & Ruzzante 2011). Although its use as a meta-N_e is not advised by Palstra and Russante (2011), we included $N_{e\ pool}$ for two reasons: 1) we wanted to verify the downward bias using our data, and 2) as a secondary estimate to compare across species in case ΣN_es was affected by reduced precision in subpopulation N_e estimates from LDNE. Local N_e estimates were generally less precise because of low sample size than N_{epool} estimates (see Results). Next we calculated the meta-N_e of Whitlock and Barton (1997; equation 23) because it explicitly accounts for the expected decrease in meta-N_e for populations experiencing extirpations and recolonizations. The Whitlock and Barton (1997) model assumes an island model with symmetric migration. Therefore, we expected meta-$N_{eW\&B}$ to be reduced relative to ΣN_es in species with asymmetric gene flow (Table 3.2). We characterized gene flow asymmetry for species by calculating the coefficient of variation of allelic richness across sites (CV[A_r], Table B.2) because gene flow asymmetry is expected to increase the variation in diversity metrics (A_r) among populations (Morrissey & de Kerckhove 2009). The fourth meta-N_e estimate used the 'bottom-up" approach developed by Tufto and Hindar (2003) [meta-$N_{eT\&H}$] that integrates a matrix of individual local N_es with patterns of connectivity expressed as a migration matrix. For each species, we defined six migration matrices to test sensitivity of the estimate to spatial configuration and *m*: 1) one-directional stepping stone model with species-specific migration rates estimated by BAYESASS, 2) island model with

migration rates estimated from BAYESASS, 3) and 4) stepping stone model with migration constant across species at $m = 0.05$ and $m = 0.1$, 5) and 6) island model with migration constant across species at $m = 0.05$ and $m = 0.1$. The eigenvalue of the resultant metapopulation matrix was minimized using the code written by J. Tufto (available from http://www.math.ntnu.no/~jarlet/migration/node4.html) in R (R Core Team 2012). A major assumption of this model is that local N_es remain constant over time, thus convergence of meta-N_e onto the eigenvalue. Yet, violations of this assumption have not previously been tested but should decrease meta-$N_{eT\&H}$ relative to ΣN_es (Palstra & Ruzzante 2011).

Statistical analyses

Our first objective was to test the hypothesis that temporal population turnover (P_e), was negatively related to standing levels of genetic diversity, ΣN_es and $N_{e\ pool}$. However, a species' genetic diversity is also expected to be positively related with abundance (McCusker & Bentzen 2010; Osborne et al. 2010). Density and P_e were not strongly correlated (Kendall's $\tau = -0.28$, $p = 0.34$) so we used multiple linear regression for the marginal effects of density and P_e to predict ΣN_es and $N_{e\ pool}$. Density, ΣN_es, and $N_{e\ pool}$ were $\log_{10}$ transformed, whereas P_e was logit transformed, to better approximate normality of residuals. To address our second objective, we compared different meta-N_e estimates relative to ΣN_es across species to evaluate if different meta-N_e models conformed to our predictions (Table 3.2) based on assumptions of different models. All analyses were performed in R 2.15.0 (R Development Core Team 2012)

Results

Estimates of N_e and $N_{e\ pool}$

Subpopulation N_es had to be estimated from lower 95% CI for several species because of the generally low precision indicated by infinite bounded estimates (Table B.3). Of the 67 species/location combinations for which subpopulation N_e was estimated using LDNE, only 21 of the estimates were finite (i.e., 95% CI not including infinity). Species with the largest proportion of finite estimates were *Gila nigra* (0.75), *Pantosteus clarkii* (0.58), and *Micropterus dolomieu* (0.50), whereas all other species only had 1 or 2 finite subpopulation N_e estimates. Estimates of $N_{e\ pool}$ from LDNE were finite with the exception of *Catostomus insignis* that had an infinite confidence interval. *Catostomus insignis*, *Agosia chrysogaster*, and *P. clarkii* had the largest ΣN_es and $N_{e\ pool}$ of all species whereas *Ameiurus natalis*, *G. nigra*, and *M. dolomieu* had the smallest (Table 3.1).

Ecological correlates of Meta-N_e

Density and P_e explained a marginally significant amount of variation of $N_{e\ pool}$ across species (adj. $r^2 = 0.42$, $p = 0.081$; Table 3.2). Density had a positive coefficient and P_e had a negative coefficient but neither were significant in the model. A significant amount of variation of ΣN_es across species was explained by density and P_e (adj. $r^2 = 0.79$, $p = 0.004$). As expected, P_e was negatively associated with ΣN_es ($\beta = -0.49$, $p = 0.028$) and density was positively associated with ΣN_es ($\beta = 0.37$, $p = 0.027$) (Figure 3.1).

Comparison of Meta-N_e Models

In general, all meta-N_e estimates decreased across species with increasing P_e and, with the exception of meta-$N_{eW\&B}$ for three species (Figure 3.2). The Whitlock and Barton (1997) estimates greatly exceeded ΣN_es for *C. insignis*, *P. clarkii*, and *A. natalis*.

The $N_{e\ pool}$ method frequently produced the lowest estimates, although meta-$N_{eW\&B}$ was equally low for three species, *G. nigra*, *Meda fulgida*, and *Tiaroga cobitis*. The Tufto and Hindar (2003) approach estimates based on the island model with species-specific *m* were very near ΣN_es in all but the two species with the lowest P_e (*C. insignis* and *P. clarkii*), in which meta-$N_{eT\&H}$ was substantially lower. The amount of differentiation within a species had a strong effect on decreasing $N_{e\ pool}$ relative to ΣN_es (Figure 3.3). As expected, the ratio of $N_{e\ pool}$: ΣN_es decreased with increasing F_{ST}. For example, *Rhinichthys osculus* had the highest F_{ST} and corresponding greatest reduction of $N_{e\ pool}$ to ΣN_es. We expected the ratio of meta-$N_{eW\&B}$: ΣN_es to decrease with increasing gene flow asymmetry as measured by CV(A_r). This prediction was generally met for species where meta-$Ne_{W\&B}$ < ΣN_es (Figure 3.3). However, the model of Whitlock and Barton (1997) is sensitive to the number of populations (*s*) (Fraser et al. 2007). We used the number of sample locations of each species for *s*, which may be overestimating the true number of distinct genetic groups of each species. For instance, halving *s* for these species reduced meta-$N_{eW\&B}$ estimates to those of $N_{e\ Pool}$ (not shown). Lastly, although we expected the ratio of meta-$N_{eT\&H}$: ΣN_es to decrease with increasing P_e, the Tufto and Hindar (2003) method was unresponsive to P_e (Figure 3.4). A brief sensitivity analysis was performed to assess the sensitivity of this estimate to spatial configuration and our estimates of *m* from BAYESASS. Generally speaking, factors that increased population isolation, either switching from island model to stepping stone, or decreasing *m* from 0.1 to 0.05 increased meta-$N_{eT\&H}$ (Figure 3.4).

Discussion

In this study, we used empirical data to examine the effect of metapopulation processes on theoretical predictions of metapopulation effective size. Genetic data of nine sympatric members of a fish metacommunity in a spatially and temporally variable riverscape were used to estimate metapopulation effective sizes based on theoretical models. First we estimated meta-N_e based on genetic data alone; the sum of each local population N_e (ΣN_es) and by pooling all individuals across locations together ($N_{e\ pool}$). Next we used estimates derived by Whitlock and Barton (1997) and Tufto and Hindar (2003) that require knowledge of population dynamics and patterns of genetic connectivity within the metapopulation system. Generalizations of our analyses of these methods under conditions of gene flow and extirpations similar to those experienced in natural populations can be summarized as follows. Temporal instability in the form of extirpations reduces meta-N_e (regardless of estimation method) across species with variable extirpation rates. However, high abundance of some species might counter the effect of extirpation. These observations conform to the general expectations that although meta-N_e is influenced by abundance, extirpations reduce meta-N_e (Whitlock and Barton 1997, Whitlock 2004). Discrepancies among the different estimation methods required further investigation. The ratio of $N_{e\ pool}$: ΣN_es decreased with increasing $F_{ST,}$ indicating downward bias of the estimate $N_{e\ pool}$ becomes more severe with population subdivision; consistent with previous findings of Palstra and Ruzzante (2011). Estimates of meta-N_e following the models of Whitlock and Barton (1997) [meta-$N_{eW\&B}$] and Tufto and Hindar (2003) [meta-$N_{eT\&H}$] were sensitive to bias in the number of subpopulations, overestimation of subpopulation N_e, and patterns of connectivity. However, our findings generally support theoretical predictions that asymmetric gene flow and variation in

population size reduce meta-N_e relative to ΣN_es (Whitlock & Barton 1997; Whitlock 2004), but the Tufto and Hindar (2003) method may have limited utility for species experiencing extirpations/recolonization dynamics. Furthermore, we discuss our results in the context of differences in life history characteristics among species and provide some implications for maintaining species' evolutionary potential that emerge from analysis.

Sources of bias in N_es

Local N_es could be biased from age-structure (i.e., overlapping generations), gene flow, and generally low precision in LDNE estimates. Ignoring age-structure could introduce downward bias of subpopulation N_e because of the introduced linkage disequilibrium resulting from sampling from multiple age-classes (Waples and Yokota 2007). Whereas, four of nine focal species have short lifespans (1-2 years), bias may be relevant for species with longer life spans (*G. nigra*, *C. insignis*, *P. clarkii*, *A. natalis*, and *M. dolomieu*). However, our sampling protocol of only sampling individuals of the same cohort (see Chapter 2 for sampling details) would have minimized the bias.

Gene flow among populations may be relevant bias of subpopulation N_e as species exhibited low global F_{ST} (all species < 0.05). Furthermore, unsampled populations within the upper Gila catchment could create bias in estimates. Species were present in locations not included in this study because our sampling design (i.e., collecting members of the same cohort) prevented us from sampling adults at locations where no juvenile individuals occurred. Gene flow from outside the system is also unlikely to influence results because the Gila River frequently dries downstream of the

study area. In addition, the linkage disequilibrium method has been reported to be fairly robust to gene flow (Waples & Do 2010). The most likely source of bias of subpopulation N_e is from low precision of LDNE estimates that resulted in having to use the lower bound 95% confidence intervals as the estimate. Whereas the precision was low, the using the lower bound estimate does provide a plausible estimate for the limit of N_e (Waples & Do 2010). We cannot rule out the possibility of bias of subpopulation N_e affecting ΣN_es and meta-$N_{eT\&H}$; however the fact that ΣN_es is strongly predicted by contemporary abundances suggests biased estimates of N_e are an unlikely explanation for the patterns we observed.

Comparison of meta-N_e among species

Despite the limitations imposed by natural levels of genetic variation, comparing meta-N_e estimates across species sheds light on potential linkages between demography, life history, and effective size. Meta-N_e estimates were positively related to contemporary relative abundances (density) and negatively related to the degree of temporal instability (P_e). Both ΣN_es and $N_{e\ pool}$ matched our expectations for species based on life history and demography. The species with the largest meta-N_e were *A. chrysogaster*, *C. insignis*, and *P. clarkii* that were the most abundant and widespread species at time of sampling (Whitney et al. 2014). Long-term surveys support high temporal stability for *C. insignis* and *P. clarkii* (Propst et al. 2008). High genetic connectivity in these species (see Chapter 2) would also contribute to these species having the largest meta-N_e of Gila River species. In contrast, factors associated with reduced meta-N_e, such as low abundance, frequent population turnover, and low genetic connectivity were exhibited by *G. nigra*, *M. fulgida*, and *M. dolomieu*, species with the

smallest meta-N_e. These results support the general notion that local extirpations and demographic fluctuations reduce meta-N_e (Wang & Caballero 1999). The effect of extirpation/recolonization dynamics for reducing meta-N_e is dependent on the number of colonists (k), and to a lesser extent, the probability of common origin (ϕ) of colonists (Whitlock & Barton 1997). Whitlock and Barton (1997) conclude that if k is small relative to local population size (N) and $\phi = 1$ (colonists are genetically similar) then extirpations should greatly reduce meta-N_e. Conversely, if k is large and $\phi = 0$ (colonizing group comprised of individuals from all populations) then the effect of extirpations should be lessened. These theoretical predictions suggest ecological tradeoffs resulting from different life history strategies may counteract the effects of extirpations. For example, *C. insignis* and *P. clarkii* are periodic strategists (large body size, long life-span, high fecundity, and low parental investment; (Olden et al. 2006)) with high temporal persistence contributing to large meta-N_e. However, *A. chrysogaster* and *M. fulgida* also exhibited high meta-N_e relative to species with similar P_e. These species are opportunistic strategists (small body, short life-span, and low fecundity) and are able to capitalize on spatially variable environments (Olden et al. 2006). If k is large for opportunistic strategists, then this could contribute to a high meta-N_e despite experiencing extirpations. Bernedonk et al. (2009) found local populations of ephemeral midges experiencing frequent extirpations to harbor less diversity than permanent midge species, but higher dispersal tendencies of ephemeral species (in numbers and distance) resulted in higher diversity at the regional scale (i.e., metapopulation) than was observed for permanent species. Although we have no estimate of k for our focal species, that some species exhibited large meta-Ne than would be expected from their P_e highlights the

importance of intrinsic traits and patterns of connectivity for shaping species' evolutionary trajectory.

Comparison of meta-N_e models

The variable, yet largely unmodified nature of the upper Gila River and its associated fish community provided an ideal system to test aspects of metapopulation and population genetic theory. We found underestimation of $N_{e\ pool}$ to increase with genetic differentiation (measured as F_{ST}) across species. Our results are in accordance with those of Palstra and Ruzzante (2011), that downward bias of $N_{e\ pool}$ can be relevant, even at low levels of genetic differentiation. Bias of $N_{e\ pool}$ might have been responsible for the lack of statistical significance in our analysis for the effects of density and $P_{e,}$ thus further emphasizing the importance of using multiple metrics for estimating N_e.

We expected the Whitlock and Barton (1997) to be the most informative meta-N_e method because it accounts for reduction in meta-N_e resulting from population extirpation/recolonization dynamics that appear to be particularly important for focal species. Therefore, violations of the assumptions of the model, such as asymmetric gene flow, should reduce the ratio of meta-$N_{eW\&B}$: ΣN_es. However, meta-$N_{eW\&B}$ was especially sensitive to *s*, and in the case of three species, produced estimates much greater than ΣN_es. Excluding these species revealed a weak negative trend between meta-$N_{eW\&B}$: ΣN_es and CV(A_r) indicating asymmetric gene flow might be influencing meta-$N_{eW\&B}$. Indeed, asymmetric gene flow has been reported to decrease meta-Ne for salmonid fishes (Kuparinen et al. 2010; Palstra & Ruzzante 2011; Gomez-Uchida et al. 2013). Analytical methods for defining true *n* from empirical data, such as Bayesian assignment tests, could

provide more informed meta-$N_{eW\&B}$ estimates to test for the effects of asymmetric gene flow on meta-$N_{eW\&B}$. Additionally, Whitlock and Barton (1997) model assumes an island model spatial configuration. The two species that exhibited the greatest reduction of meta-$N_{eW\&B}$, *M. fulgida* and *R. osculus*, also displayed patterns of genetic variation consistent with regional structuring (Chapter 2). The effects of different spatial configurations on meta-$N_{eW\&B}$ have yet to be thoroughly investigated.

Lastly, we considered assumptions of the eigenvalue variance N_e developed by Tufto and Hindar (2003); constant subpopulation sizes and fixed migration pattern. Because these key assumptions were violated for all focal species, we expected the ratio meta-$N_{eT\&H}$: ΣN_es to decrease with increasing temporal instability. This expectation was not met as meta-$N_{eT\&H}$ was unresponsive to P_e and produced similar estimates to ΣN_es for all but *C. insignis* and *P. clarkii*. Meta-$N_{eT\&H}$ was greatly reduced in these species owing to two tributary populations with exceptionally low N_es (Chapter 2). Large variance across subpopulation N_es causes some subpopulations to exert stronger influence than others reducing total eigenvalue effective size, even in the absence of asymmetric gene flow (Tufto & Hindar 2003). Meta-$N_{eT\&H}$ was only informative for the two species with the lowest P_e suggesting it may have limited applicability for species with extreme demographic fluctuations. However, meta-$N_{eT\&H}$ was sensitive to spatial configuration and *m* and uncertainty of our *m* estimates could be influencing meta-N_e. Increasing population isolation, either by decreasing *m* or by spatial configuration as in the case of island model to stepping stone model, increases meta-$N_{eT\&H}$ (Tufto & Hindar 2003). In principle, it is possible to define a migration matrix using the unidirectional migration rates from BAYESASS, but when applied using our data, led to infinite meta-

N_e for all species. Hence, we used mean unidirectional estimates. Despite uncertainty in empirically derived *m*, meta-$N_{eT\&H}$ appears to have limited functionality for species experiencing extirpation/recolonization dynamics.

In conclusion, by incorporating demographic and genetic data from nine distantly related, sympatric species, we were able to test the effects of nonequilibrium processes on species' evolutionary trajectories. Our results provide much needed empirical support for the theoretical predictions that extirpation/recolonization dynamics decrease metapopulation effective size but the magnitude of the effect depends on ecological characteristics related to dispersal (Whitlock & McCauley 1990; Whitlock & Barton 1997). Furthermore, different life histories can lead to trade-offs in the regulation of genetic diversity. For example, our results and those of Berendonk et al. (2009) suggest the trade-offs related to life history (opportunistic vs. periodic, ephemeral vs. permanent) can have different consequences on evolutionary potential that are related to patterns of genetic connectivity. These results have direct conservation implications for maintaining genetic diversity of species with different life histories. For species exhibiting high temporal stability, the primary factor responsible for reduced meta-N_e is variance among local N_es. Minimizing among population variance will slow the decay of genetic diversity. Alternatively, meta-Ne of species exhibiting population turnover is affected by number of colonizers. Therefore, maintaining dispersal corridors to allow for maximum number of colonists will help to minimize the decay of genetic diversity.

Literature Cited

Berendonk TU, Spitze K, Kerfoot WC (2009) Ephemeral metapopulations show high genetic diversity at regional scales. Ecology, 90, 2670-2675.

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to Conservation Genetics, Second edn. Cambridge University Press, New York.

Fraser DJ, Hansen MM, Ostergaard S, Tessier N, Legault M, Bernatchez L (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. Molecular Ecology, 16, 3866-3889.

Gomez-Uchida D, Palstra FP, Knight TW, Ruzzante DE (2013) Contemporary effective population and metapopulation size (N_e and meta-N_e): comparison among three salmonids inhabiting a fragmented system and differing in gene flow and its asymmetries. Ecology and Evolution, 3, 569-580.

Ingvarsson PK, Olsson K, Ericson L (1997) Extinction-recolonization dynamics in the mycophagous beetle *Phalacrus substriatus*. Evolution, 51, 187-195.

Kuparinen A, Tufto J, Consuegra S, Hindar K, Merila J, de Leaniz C (2010) Effective size of an Atlantic salmon (*Salmo salar* L.) metapopulation in Northern Spain. Conservation Genetics, 11, 1559-1565.

Maruyama T (1970) Analysis of population structure. I. One-dimensional stepping-stone models of finite length. Annals of Human Genetics, 34, 201-219.

Maruyama T (1971) Analysis of population structure. II. Two-dimensional stepping stone models of finite length and other geographically structured populations. Annals of Human Genetics, 35, 179-196.

McCusker MR, Bentzen P (2010) Positive relationships between genetic diversity and abundance in fishes. Molecular Ecology, 19, 4852-4862.

Morrissey MB, de Kerckhove DT (2009) The maintenance of genetic variation due to asymmetric gene flow in dendritic metapopulations. American Naturalist, 174, 875-889.

Nunney L (1999) The effective size of a hierarchically structured population. Evolution, 53, 1-10.

Olden JD, Poff NL, Bestgen KR (2006) Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. Ecological Monographs, 76, 25-40.

Osborne MJ, Davenport SR, Hoagstrom CW, Turner TF (2010) Genetic effective size, N_e, tracks density in a small freshwater cyprinid, Pecos bluntnose shiner (*Notropis simus pecosensis*). Molecular Ecology, 19, 2832-2844.

Palstra FP, Ruzzante DE (2011) Demographic and genetic factors shaping contemporary metapopulation effective size and its empirical estimation in salmonid fish. Heredity, 107, 444-455.

Propst DL, Gido KB, Stefferud JA (2008) Natural flow regimes, nonnative fishes, and native fish persistence in arid-land river systems. Ecological Applications, 18, 1236-1252.

R Core Team (2012) R: A language and environment for statistical computing. Vienna, Austria.

Ray C (2001) Maintaining genetic diversity despite local extinctions: effects of population scale. Biological Conservation, 100, 3-14.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. Trends in Ecology & Evolution, 11, 338-342.

Tufto J, Hindar K (2003) Effective size in management and conservation of subdivided populations. Journal of Theoretical Biology, 222, 273-281.

Wang JL, Caballero A (1999) Developments in predicting the effective size of subdivided populations. Heredity, 82, 212-226.

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources, 8, 753-756.

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications, 3, 244-262.

Whitlock MC (2004) Selection and drift in metapopulations. Pages 153-175 *in* Hanski I, Gaggiotti OE, editors. Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, San Diego, CA.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. Genetics, 146, 427-441.

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, 44, 1717-1724.

Whitney JE, Gido KB, Pilger TJ, Propst DL, Turner TF (2015) Metapopulation analysis indicates native and non-native fishes respond differently to effects of wildfire on desert streams. Ecology of Freshwater Fish.

Whitney JE, Gido KB, Propst DL (2014) Factors associated with the success of native and nonnative species in an unfragmented arid-land riverscape. Canadian Journal of Fisheries and Aquatic Sciences, 71, 1134-1145.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics, 163, 1177-1191.

Wright S (1931) Evolution in Mendelian populations. Genetics, 16, 0097-0159.

Wright S (1943) Isolation by distance. Genetics, 28, 114-138.

Wright S (1946) Isolation by distance under diverse systems of mating. Genetics, 31, 39-59.

Tables and Figures

Table 3.1 Ecological and genetic parameters used to estimate different meta-N_e models in the warm-water fish community of the Gila River, NM, USA. Reported values include number of sample locations (*s*), total number of individuals sampled (*N*), probability of extirpation (P_e), global differentiation (F_{ST} from Chapter 2), meta-N_e estimates from all individuals pooled ($N_{e\ pool}$) and by summing subpopulation N_es (ΣN_es), and migration rate (*m*) estimated using the program BAYESASS (Wilson & Rannala 2003).

Species	*s*	*N*	Density (m^{-2})	P_e	F_{ST} (95% CI)	N_{epool} (95% CI)	ΣN_es	*m* ± SE
A. chrysogaster	13	397	0.043	0.13	0.016 (0.012-0.020)	1217 (595-33322)	1997.1 (69.9-326.1)	0.024 ± 0.004
G. nigra	4	80	0.004	0.35	0.027 (0.014-0.041)	86 (62-131)	180.9 (8.2-61.5)	0.076 ± 0.029
M. fulgida	6	170	0.067	0.19	0.021 (0.015-0.026)	325 (244-470)	678.6 (50.3-178.7)	0.052 ± 0.016
R. osculus	8	227	0.056	0.08	0.042 (0.027-0.062)	143 (99-224)	1281.2 (88.7-314.7)	0.037 ± 0.009
T. cobitis	4	112	0.011	0.15	0.008 (0.005-0.011)	602 (292-20719)	888.9 (140.6-349.3)	0.069 ± 0.027
C. insignis	9	292	0.033	0.01	0.014 (0.010-0.020)	1617 (605-inf)	2299.9 (9.5-724.8)	0.032 ± 0.008

Species	*s*	*N*	Density (m^{-2})	P_e	F_{ST} (95% CI)	N_{epool} (95% CI)	$\Sigma N_e s$	*m* ± SE
P. clarkii	12	344	0.021	0.06	0.013 (0.009-0.016)	496 (331-907)	1773.1 (34.6-422.8)	0.027 ± 0.004
A. natalis	5	122	0.003	0.20	0.007 (-0.004-0.024)	121 (44-2649)	232.8 (16.8-100.4)	0.069 ± 0.008
M. dolomieu	6	195	0.001	0.17	0.036 (0.024-0.048)	116 (58-330)	307.8 (12.3-155.9)	0.036 ± 0.004

Table 3.2 Models for estimating effective metapopulation size (meta-N_e), the size of an idealized subdivided population experiencing the same rate of inbreeding as the population under study.

Name	Model*	Expectations	Reference
Island	meta-$N_e = \frac{\sum_{i=1}^{S} N_{ei}}{1 - F_{ST}}$	meta-N_e > ΣN_es unless F_{ST} = 0; inequality increases with F_{ST}	Wright (1943)
Extirpation-recolonization	meta-$N_e = \frac{s}{4(m+e)F_{ST}}$	meta-N_e < ΣN_es; inequality increases with asymmetric gene flow	Whitlock and Barton (1997)
Bottom-up	meta-$N_e = \frac{1}{2-2\lambda_1}$	meta-N_e < ΣN_es; inequality increases with temporal variance in abundance or extirpation	Tufto and Hindar (2003)

* Metapopulation parameters: *s* = number of subpopulations, N_{ei} = effective size of subpopulation *i*, FST = global differentiation among all subpopulations, *m* = average unidirectional rate between subpopulations, *e* = average probability of extirpation across all subpopulations, λ_1 = dominant eigenvalue derived from a matrix of pairwise migration rates between subpopulations (**M**) and vector of subpopulation N_e values (**N**).

Table 3.3 Multiple linear regression results for predicting meta-N_e of pooled samples ($N_{e\ pool}$) and by summing local N_es (ΣN_es) of nine fish species from the Gila River, NM, USA.

	Betas			Model		
N_{epool}	Estimate	SE	*P*	Adj. r^2	$F_{(2,6)}$	*P*
(Intercept)	2.55	0.634	0.007	0.42	3.93	0.081
P_e	-0.53	0.31	0.145			
Density	0.28	0.24	0.29			

	Betas			Model		
ΣN_es	Estimate	SE	*P*	Adj. r^2	$F_{(2,6)}$	*P*
(Intercept)	3.14	0.34	> 0.001	0.79	16.11	0.004
P_e	-0.49	0.17	0.028			
Density	0.37	0.13	0.027			

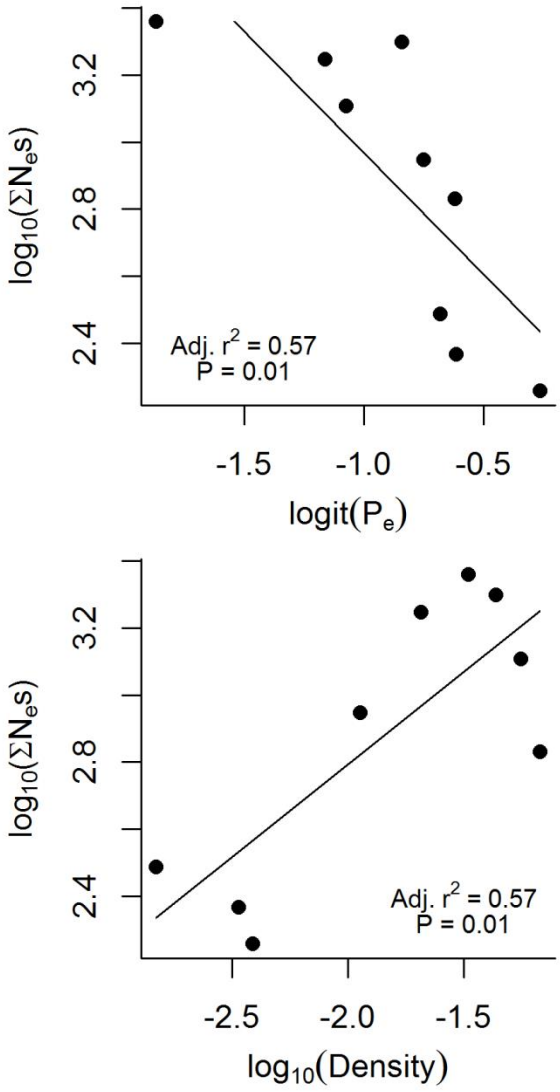

Figure 3.1 Univariate relationships between $\Sigma N_e s$ and P_e (top) and contemporary density of nine fish species from the Gila River, NM.

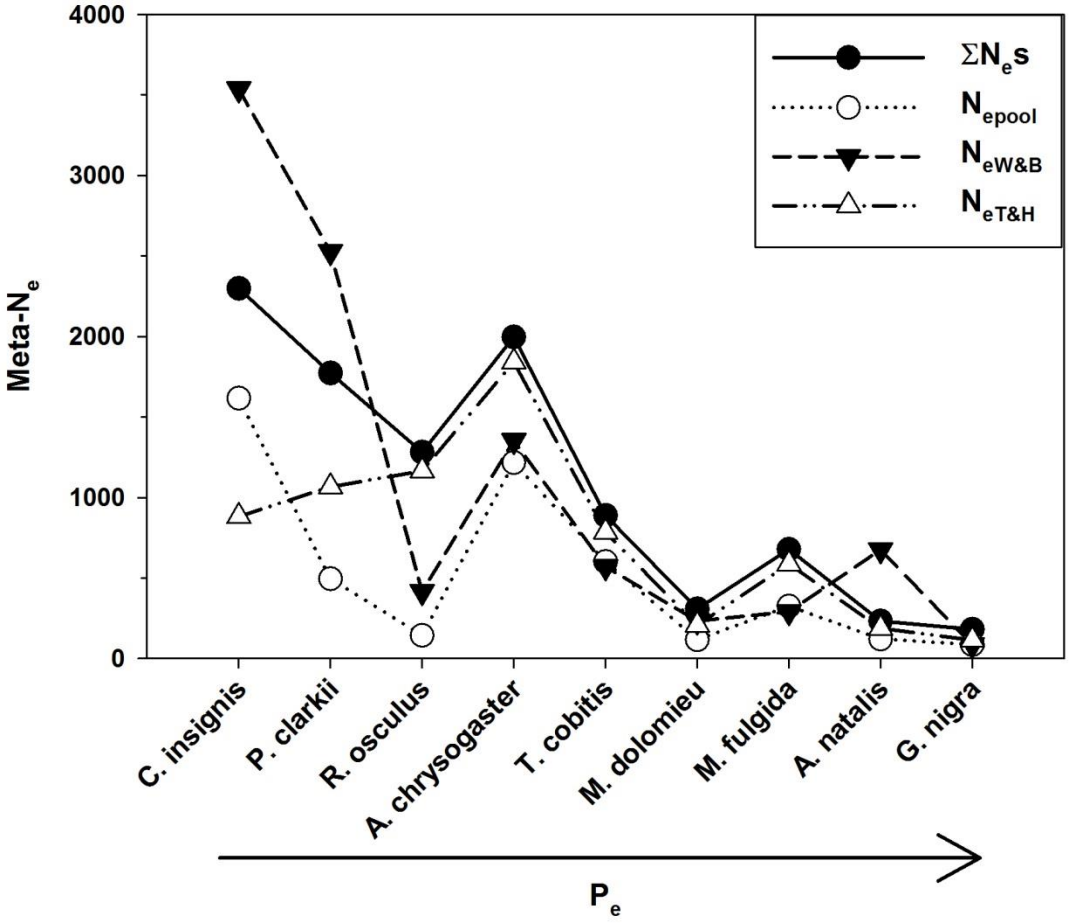

Figure 3.2 Estimates of meta-N_e for each of nine Gila River fish species ranked on P_e. Meta- N_e estimated as $\Sigma N_e s$ (null-model) were used to compare all other meta-Ne estimates; $N_{e\ pool}$, and two estimates based on models with different assumptions

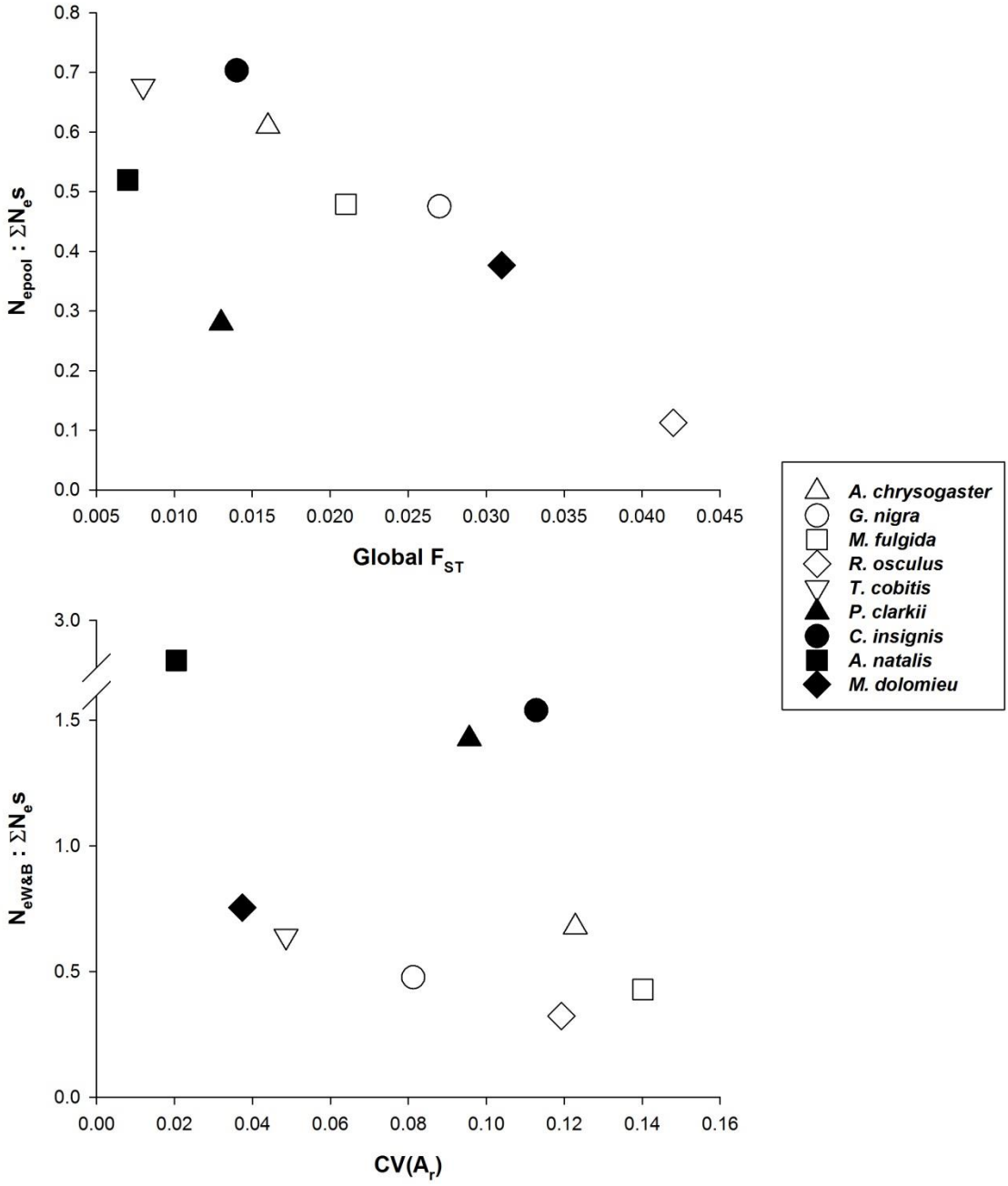

Figure 3.3 Top panel: The effect of increasing genetic differentiation on the ratio $N_{e\ pool}$: $\Sigma N_e s$ across nine species of Gila River fishes to evaluate the behavior of $N_{e\ pool}$ as an estimate of meta-N_e. Bottom panel: The effect of increasing gene flow asymmetry (i.e., CV[A_r]) on meta-N_e derived from the method of Whitlock and Barton (1997) relative to $\Sigma N_e s$. Note the break in scale on the y-axis.

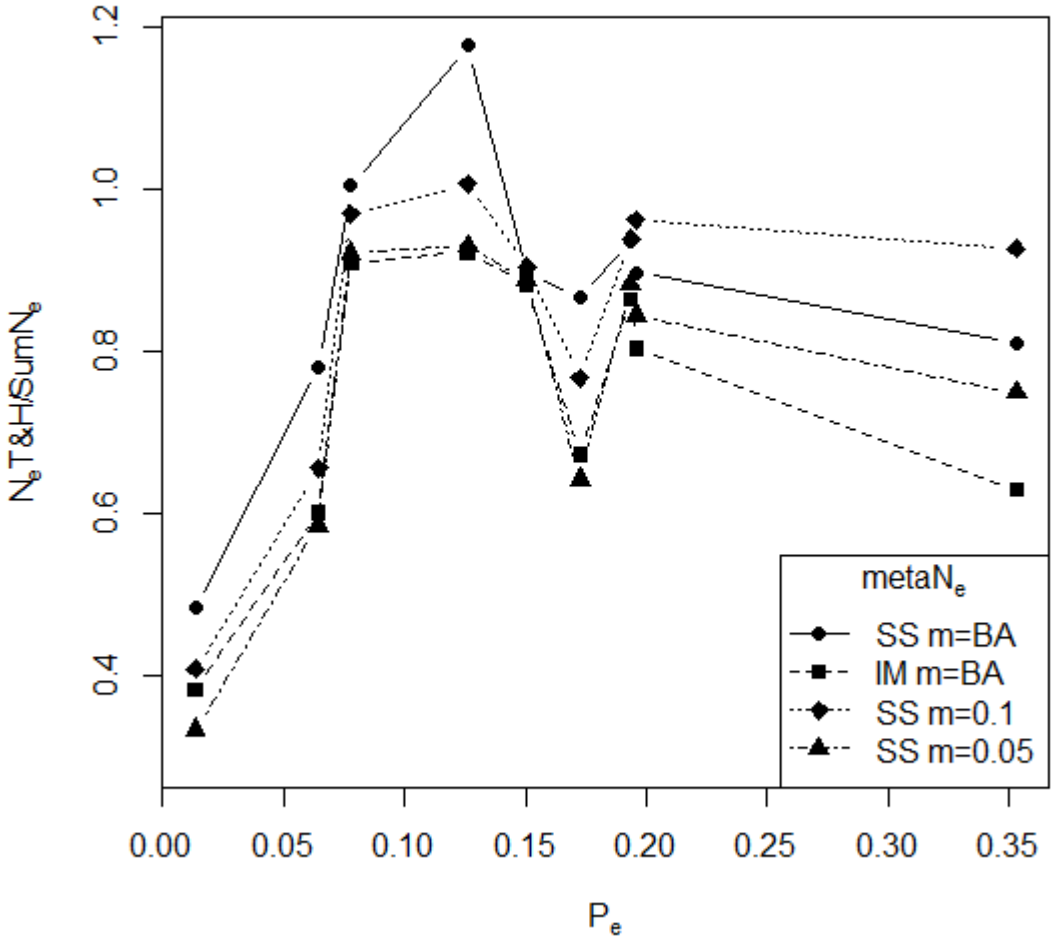

Figure 3.4 Evaluating Tufto and Hindar (2003) meta-N_e estimate versus extirpation (P_e). Generally, extirpation has little effect on the estimate (order of species on x-axis same as Figure 3.2). Altering gene flow (*m*) or spatial configuration (stepping stone model [SS] or island model [IM]) can have variable effects on meta-N_e relative to ΣN_es. Lines and symbols used to group parameterizations of the spatially explicit migration matrix (SS or IM) with rate *m* = species-specific rate from BAYESASS (Table 3.1), 0.1 or 0.05 for each species.

SUMMARY

This body of research incorporates ecological monitoring and multi-species genetic data affording a unique opportunity to address linkages between species ecology (via life history and dispersal) and patterns of genetic variation. Chapter 1 takes a conservation genetic approach to address key concerns for the genetic health of three Federal and State protected species of the upper Gila River, NM. Chapter 2 broadens the scope of focal species with vastly different life histories to evaluate how dispersal and life history influence patterns of genetic structure within a shared riverscape. Chapter 3 examines the relationship between metapopulation processes and species evolutionary trajectories. Whereas Chapters 2 and 3 are relevant to expanding knowledge of evolutionary ecology, all three chapters provide apposite and timely conservation implications for the imperiled native fish fauna of the upper Gila River.

Although one of the last remaining, free-flowing rivers in North America, the upper Gila River is not immune from succumbing to same fate as others. The upper Gila already hosts a suite of nonnative species including predators known to prey on native fishes (Pilger et al. 2010). The Arizona Water Settlements Act (AWSA) of 2004 has apportioned federal funds for water development projects, some of which would result in fragmentation to the upper Gila River (O'Leary 2013). Furthermore, climate change induced shifts to wildfire regimes (increased intensity and frequency) disproportionately affect native fishes, limiting their success in the presence of nonnative predators (Whitney 2014). A primary motivation of this body of research was to provide insights as to how these conservation threats will alter patterns of genetic connectivity and diversity. Key findings were that Gila River fishes generally exhibited a high degree of genetic

connectivity as indicated by relatively low F_{ST}. Maintaining functional connectivity will be important for limiting the reduction of genetic diversity for all species in this imperiled system. However, because distance has a stronger isolating effect on low fecundity species (*Agosia chrysogaster*, *Meda fulgida. Rhinichthys osculus* and *Tiaroga cobitis*), they are most likely to experience rapid declines in genetic diversity if connectivity is reduced (Chapters 1 and 2). Alternatively, for the more fecund native species (*Gila nigra*, *Catostomus insignis*, and *Pantosteus clarkii*), factors associated with reducing local N_e, such as negative interactions with nonnative species or disturbance, are most likely to increase the overall effect of drift across populations, thereby reducing metapopulation genetic effective size (Chapters 2 and 3). These results uphold the conservation actions recommended by previous research that maintaining connectivity of the upper Gila River system and targeted nonnative removal at specific problem locations are currently the best actions for ensuring native persistence in the face of altered disturbance regimes (Propst et al. 2008; Pilger et al. 2010; Propst et al. 2014; Whitney et al. 2014; Pilger et al. 2015; Whitney et al. 2015).

Chapters presented here also contribute insight into how ecology influences evolutionary dynamic of species, and represents one of only a handful of studies linking metapopulation dynamics to species genetic attributes. Chapter 2 uses combined genetic and ecological data to evaluate the relationship between intrinsic differences among species and differential patterns of genetic connectivity. Patterns of genetic connectivity varied across species and were related to species-specific dispersal and population size. Testing for effects of life-history traits on levels of genetic differentiation across species revealed equivocal results for egg size as a predictor of global F_{ST} across species. This

result contrasts with that of Turner and Trexler (1998) who found egg size and female body size to be strongly associated with levels of levels of gene flow among several darter species. However, patterns of spatial genetic structure across species, as measured by Mantel's *r*, were strongly associated with fecundity, suggesting that life history traits might influence how species interact with the landscape.

The species-specific patterns of genetic connectivity observed in Chapter 2 provided the ecological basis for understanding the effects of metapopulation dynamics on species genetic diversity, the focus of Chapter 3. Genetic and ecological data were incorporated into meta-N_e using four separate models that account for different demographic processes and spatial configurations. Temporal instability in the form of extirpations reduced meta-N_e (regardless of estimation method) across species with variable extirpation rates. However, high abundance of some species could counter the effect of extirpation. Our results support the conclusion that trade-offs related to life history strategies could lead to different consequences for maintaining genetic diversity of species. In addition, we evaluated different meta-N_e methods and found they may be informative for identifying demographic processes affecting meta-N_e, such as asymmetric gene flow and variance among local N_e. But models were sensitive to different assumptions, and thus await further investigation.

Literature Cited

O'Leary M (2013) Damming a river to save it. Evironmental Flows Bulletin December 2013: 6-10.

Pilger TJ, Gido KB, Propst DL (2010) Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: implications for native fish conservation. Ecology of Freshwater Fish, 19, 300-321.

Pilger TJ, Gido KB, Propst DL, Whitney JE, Turner TF (2015) Comparative conservation genetics of protected endemic fishes in an arid-land riverscape. Conservation Genetics, 1-14.

Propst DL, Gido KB, Stefferud JA (2008) Natural flow regimes, nonnative fishes, and native fish persistence in arid-land river systems. Ecological Applications, 18, 1236-1252.

Propst DL, Gido KB, Whitney JE, Gilbert EI, Pilger TJ, Monié AM, Paroz YM, Wick JM, Monzingo JA, Myers DM (2014) Efficacy of mechanically removing nonnative predators from a desert stream. River Research and Applications DOI: 10.1002/rra.2768.

Turner TF, Trexler JC (1998) Ecological and historical associations of gene flow in darters (Teleostei: Percidae). Evolution, 52, 1781-1801.

Whitney JE (2014) Spatiotemporal response of aquatic native and nonnative taxa to wildfire disturbance in a desert stream network. Kansas State University

Whitney JE, Gido KB, Pilger TJ, Propst DL, Turner TF (2015) Metapopulation analysis indicates native and non-native fishes respond differently to effects of wildfire on desert streams. Ecology of Freshwater Fish. DOI: 10.1111/eff.12217.

Whitney JE, Gido KB, Propst DL (2014) Factors associated with the success of native and nonnative species in an unfragmented arid-land riverscape. Canadian Journal of Fisheries and Aquatic Sciences, 71, 1134-1145.

Appendix A APPENDIX FOR CHAPTER 1

Table A.1 Summary of genetic screening and cross-species amplification of microsatellite loci for Gila River imperiled fishes collected in 2009 and 2010. Included are number of alleles (N_A), size range of alleles in base pairs (bp), optimal annealing temperature (T_A), gene diversity (H_E) and observed heterozygosity (H_O). Superscripted numbers following locus name indicate loci combined for multiplex reactions. PCR temperature profiles consisted of 90 °C for 3 min initial denaturation followed by 30 cycles of 90 °C for 30 sec, T_A for 30 sec, and 72 °C for 45 sec, and ending with a final extension at 72 °C for 15 min.

Locus	N_A	Size Range (bp)	T_A (°C)	H_E	H_O	DYE	Reference
Headwater chub							
Ca6	5	191-207	49	0.50	0.27	FAM	Dimsoski et al. 2000
Ca8	24	140-264	49	0.92	0.97	FAM	Dimsoski et al. 2000
Cyp2 [1]	4	173-191	53	0.41	0.45	HEX	Baerwald and May 2004
Cyp45 [1]	5	105-129	53	0.13	0.13	FAM	Baerwald and May 2004
Cyp5	17	152-222	60	0.87	0.89	FAM	Baerwald and May 2004
Gel36	6	193-215	50	0.68	0.77	HEX	Keeler-Foster et al. 2004
Lep27-8	12	149-205	56	0.88	0.92	FAM	Mock et al. 2008
Lep28-7 [1]	20	294-414	52	0.91	0.92	FAM	Mock et al. 2008
Rhca15 [2]	8	156-182	63	0.74	0.65	FAM	Girard & Angers 2006
Rhca20 [2]	3	101-113	62	0.25	0.18	HEX	Girard & Angers 2006
Spikedace							
ParB5T [2]	28	157-273	49	0.94	0.84	FAM	Vu et al. 2005
ParB68 [2]	13	294-346	49	0.83	0.71	HEX	Vu et al. 2005
ParB3OT [1]	11	200-244	50	0.79	0.76	FAM	Vu et al. 2005

Locus	N_A	Size Range (bp)	T_A (°C)	H_E	H_O	DYE	Reference
ParB56MB [1]	23	281-369	50	0.91	0.86	FAM	Vu et al. 2005
ParB64ML [1]	21	233-333	50	0.91	0.80	HEX	Vu et al. 2005
ParC55TR [3]	23	203-295	53	0.91	0.93	FAM	Vu et al. 2005
Nme93 [3]	13	99-141	53	0.67	0.30	FAM	Gold et al. 2004
Nme232 [3]	14	224-262	53	0.68	0.74	HEX	Gold et al. 2004
Lep4-6	10	158-194	60	0.63	0.63	FAM	Mock et al. 2008
Lep28-7	14	225-277	48	0.77	0.78	FAM	Mock et al. 2008
Loach minnow							
Ach3 [1]	4	131-137	60	0.14	0.15	HEX	Trujillo et al. 2012
Ach19	7	134-150	52	0.65	0.66	FAM	Trujillo et al. 2012
Cyp5	24	211-359	49	0.92	0.95	FAM	Baerwald and May 2004
Lco8 [1]	37	277-361	60	0.95	0.96	FAM	Turner et al. 2004
Lep4-6 [1]	18	171-215	60	0.90	0.87	FAM	Mock et al. 2008
Ppro126 [2]	2	154-156	61.5	0.50	0.46	FAM	Bessert & Orti 2003
Ppro132	15	112-162	60	0.84	0.82	FAM	Bessert & Orti 2003
Rhca15	56	165-455	55	0.96	0.51	FAM	Girard & Angers 2006
Rhca20 [2]	6	105-121	60	0.53	0.53	HEX	Girard & Angers 2006
Rhca24	25	286-362	48	0.91	0.86	HEX	Girard & Angers 2006

References for Table A.1

Baerwald, M.R. & May, B. 2004. Characterization of microsatellite loci for five members of the minnow family Cyprinidae found in the Sacramento-San Joaquin Delta and its tributaries. Molecular Ecology Notes 4: 385-390.

Bessert, M.L. & Orti, G. 2003. Microsatellite loci for paternity analysis in the fathead minnow, *Pimephales promelas* (Teleostei: Cyprinidae). Molecular Ecology Notes 3: 532-534.

Burridge, C.P. & Gold, J.R. 2003. Conservation genetic studies of the endangered Cape Fear Shiner, *Notropis mekistocholas* (Teleostei: Cyprinidae). Conservation Genetics 4: 219-225.

Dimsoski, P., Toth, G. P. & Bagley, M.J. 2000. Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). Molecular Ecology 9: 2187-2189.

Girard, P. & Angers, B. 2006. Characterization of microsatellite loci in longnose dace (*Rhinichthys cataractae*) and interspecific amplification in five other Leuciscinae species. Molecular Ecology Notes 6: 69-71.

Keeler-Foster, C.L., Spies, I.B., Bondu-Hawkins, V. & Bentzen, P. 2004. Development of microsatellite markers in bonytail (*Gila elegans*) with cross-species amplification in humpback chub (*Gila cypha*). Molecular Ecology Notes 4: 23-25.

Mock, K.E., Bjerregaard, L.S., Belk, M.C., Rowe, C. & Johnson, B.J. 2008. Microsatellite markers for leatherside chubs *Lepidomeda aliciae* and *Lepidomeda copei*. Molecular Ecology Resources 8: 172-174.

Trujillo, J.D., T.J. Pilger, M.R. Douglas, M.E. Douglas, and T.F. Turner. 2012. Microsatellite markers for Longfin Dace, *Agosia chrysogaster*, a sentinel fish species in imperiled arid-land rivers of Sonoran Desert. Conservation Genetics Resources 4: 927-929.

Turner, T.F., Dowling, T.E., Broughton, R.E., et al. 2004. Variable microsatellite markers amplify across divergent lineages of cyprinid fishes (subfamily Leusicinae). Conservation Genetics 5: 279-281.

Vu, N.V., Keeler-Foster, C.L., Spies, I.B. & Ribeiro, D.T. 2005. Twelve microsatellite markers developed in woundfin (Plagopterus argentissimus), an endangered warmwater fish of the lower Colorado River basin. Molecular Ecology Notes 5: 302-304.

Table A.2 Pairwise F_{ST} values for three protected Gila River warm-water fishes sampled in 2009 and 2010. Headwater chub was only found at two locations in 2009, thus no pairwise table.

Headwater Chub

2010	WF1	MF	EF	GM1
WF1	0			
MF	0.028**	0		
EF	0.018**	0.023**	0	
GM1	0.017*	0.040**	0.037*	0

Spikedace

2009	WF2	GM2	GM3
WF2	0		
GM2	0.031**	0	
GM3	0.037**	-0.002	0

2010	WF1	MF	WF2	GM2	GM3	GM4
WF1	0					
MF	0.006	0				
WF2	0.013*	-0.002	0			
GM2	0.042**	0.033**	0.036**	0		
GM3	0.032**	0.027**	0.027**	0.007*	0	
GM4	0.036**	0.032**	0.032**	0.005	-0.002	0

Loach Minnow

2009	GM1	GM2	GM3
GM1	0		
GM2	0.024**	0	
GM3	0.021**	-0.002	0

2010	GM1	GM2	GM3	GM4
GM1	0			
GM2	0.011*	0		
GM3	0.014**	0.004	0	
GM4	0.015*	0.005	0.005	0

*Significant at 0.05 level

**Significant at < 0.001 level

Appendix B APPENDIX FOR CHAPTERS 2 AND 3

Table B.1 Summary of genetic screening and cross-species amplification of microsatellite loci for Gila River imperiled fishes collected in 2009 and 2010. Included are number of alleles (N_A), size range of alleles in base pairs (bp), optimal annealing temperature (T_A), gene diversity (H_E) and observed heterozygosities (H_O). Superscripted numbers following locus name indicate loci combined for multiplex reactions. PCR temperature profiles consisted of 90 °C for 3 min initial denaturation followed by 30 cycles of 90 °C for 30 sec, T_A for 30 sec, and 72 °C for 45 sec, and ending with a final extension at 72 °C for 15 min.

Locus	N_A	Size Range (bp)	T_A (°C)	H_E	H_O
A. chrysogaster					
Ach1	27	130-236	56.5	0.93	0.93
Ach3	30	131-201	56.5	0.87	0.71
Ach19	7	119-133	56.5	0.67	0.63
Ach21	7	96-116	56.5	0.67	0.70
Ach39	12	70-104	65	0.30	0.30
Ach61	11	190-218	56.5	0.72	0.74
Lco3	16	226-256	53	0.84	0.83
Lco6	20	164-206	51	0.87	0.88
Nme93	13	68-114	54	0.78	0.79
Nme232	36	237-333	58	0.93	0.95
R. osculus					
Rhca20	30	106-164	60	0.88	0.68
Rhca23	28	272-366	61.5	0.88	0.88
Lco1	39	234-366	49	0.95	0.90
Lco3	5	235-247	64	0.33	0.26
Lco4	16	216-276	60	0.75	0.76
Lco7	8	136-152	49	0.72	0.73
Lco8	17	270-396	60	0.76	0.71
Ppro126	6	152-166	64	0.52	0.54
Ppro132	4	114-130	64	0.44	0.33
Nme93	19	78-126	55	0.83	0.81
T. cobitis					
Ach3	4	131-137	60	0.14	0.15
Ach19	7	134-150	52	0.65	0.66
Cyp5	24	211-359	49	0.92	0.95

Locus	N_A	Size Range (bp)	T_A (°C)	H_E	H_O
Lco8	37	277-361	60	0.95	0.96
Lep4.6	18	171-215	60	0.90	0.87
Ppro126	2	154-156	61.5	0.50	0.46
Ppro132	15	112-162	60	0.84	0.82
Rhca15	56	165-455	55	0.96	0.51
Rhca20	6	105-121	60	0.53	0.53
Rhca24	25	286-362	48	0.91	0.86
P. clarkii					
Dlu209	18	150-194	50	0.82	0.78
Dlu229	15	126-160	58	0.81	0.79
Dlu230	10	107-133	50	0.85	0.81
Dlu233	11	124-162	58	0.33	0.31
Dlu456	24	162-294	54	0.90	0.89
Dlu4153	28	152-272	58	0.93	0.96
Dlu4184	19	173-249	58	0.89	0.90
Xte9	14	156-220	52	0.67	0.67
Xte11	29	272-334	50	0.90	0.89
Xte17	9	198-228	52	0.61	0.61
C. insignis					
Dlu209	15	150-190	50	0.60	0.63
Dlu229	17	122-162	58	0.69	0.69
Dlu233	18	128-182	60	0.77	0.78
Dlu456	28	158-298	54	0.94	0.94
Dlu4153	13	164-212	58	0.88	0.87
Dlu4184	15	173-237	58	0.89	0.91
Xte9	24	156-228	55	0.85	0.87
Xte11	18	272-318	50	0.89	0.91
Xte17	8	192-216	52	0.74	0.75
A. natalis					
An7	3	171-179	0.64	0.64	
An11	3	177-217	0.53	0.68	
An12	2	136-140	0.23	0.21	
An13	3	237-257	0.02	0.02	
An17	2	146-154	0.41	0.40	
Amn16	2	97-103	0.05	0.05	
Amn34	2	187-197	0.02	0.02	
Amn42	3	143-153	0.25	0.27	
Amn44	7	141-153	0.69	0.70	
M. dolomieu					

Locus	N_A	Size Range (bp)	T_A (°C)	H_E	H_O
Mdo1	4	199-207		0.47	0.45
Mdo2	3	196-200		0.57	0.59
Mdo3	4	100-132		0.35	0.31
Mdo5	3	198-204		0.40	0.43
Mdo6	2	146-148		0.47	0.34
Mdo7	2	164-168		0.49	0.45
Mdo8	4	213-221		0.44	0.46
Mdo9	5	119-133		0.62	0.66
Mdo10	2	99-105		0.35	0.31
Mdo11	3	169-173		0.51	0.50

Table B.2 Genetic summary statistics for fishes of the upper Gila River catchment sampled from 2010. At each site, sample size (n), gene diversity (H_E), observed heterozygosity (H_O), allelic richness (A_R), inbreeding coefficient (F_{IS}), and mean Queller and Goodnight (1989) estimator of relatedness (r) are reported. Missing values (indicated with a dash) were not estimated due to small sample size

Species	Site	n	H_E	H_O	A_R	F_{IS}
A. chrysogaster						
	BC	30	0.7208	0.6926	7.8037	0.0400
	WF	36	0.7151	0.6356	8.232	0.113
	MF	30	0.7220	0.7449	8.7124	-0.0320
	HB	30	0.7448	0.7244	9.093	0.0280
	GV	28	0.7227	0.7028	8.1194	0.0280
	SC	30	0.7701	0.7331	8.9954	0.05
	TC	30	0.7660	0.8007	9.8816	-0.05
	GF	31	0.7750	0.7478	10.7339	0.036
	RS	30	0.7883	0.7829	10.6204	0.007
	BA	32	0.7831	0.8028	10.7153	-0.026
	MB	29	0.7819	0.7668	11.2122	0.02
	BlueC	30	0.7721	0.7837	10.0902	-0.015
	SSC	31	0.7988	0.7803	10.853	0.024
G. nigra						
	UEF	19	0.6376	0.6286	5.8518	0.0150
	WF	26	0.6443	0.6266	5.9226	0.0280
	MF	20	0.6007	0.5618	4.9590	0.0670
	HB	3	0.6233	0.6167		
	GV	12	0.6292	0.6462	5.3818	-0.03
M. fulgida						
	WF	30	0.7547	0.6567	8.2236	0.132
	MF	34	0.7877	0.6989	8.4258	0.1150
	HB	30	0.7605	0.6877	7.8398	0.0980
	RS	28	0.8412	0.8018	10.371	0.0480

Species	Site	n	H_E	H_O	A_R	F_{IS}
	BA	31	0.8459	0.7730	10.5774	0.0870
	MB	17	0.8365	0.7837	10.6252	0.07
R. osculus						
	UEF	19	0.6920	0.6365	8.3194	0.082
	BC	30	0.7196	0.6733	9.3680	0.065
	WF	26	0.7035	0.6731	8.7651	0.0440
	MF	30	0.7024	0.6783	8.3561	0.0350
	HB	30	0.7323	0.6821	8.4696	0.07
	LC	32	0.7056	0.6546	7.5397	0.07
	GV	30	0.7120	0.6472	8.5606	0.092
	BlueC	30	0.6750	0.6427	6.1222	0.049
T. cobitis						
	GV	30	0.7254	0.6436	9.95	0.115
	RS	29	0.7394	0.7267	11.0374	0.0170
	BA	34	0.7156	0.6428	10.7631	0.1030
	MB	19	0.7414	0.6944	10.1545	0.0650
P. clarkii						
	UEF	32	0.7678	0.7746	8.7506	-0.0090
	BC	35	0.7607	0.7287	8.0373	0.04
	WF	30	0.7800	0.7597	9.2113	0.027
	MF	32	0.7751	0.7652	9.0886	0.0130
	HB	31	0.7814	0.7441	8.976	0.0480
	LC	31	0.7910	0.8082	8.1408	-0.0220
	LEF	29	0.7506	0.7276	8.6685	0.03
	GV	29	0.8114	0.7914	10.1016	0.025
	GF	14	0.7429	0.7500	7.2	-0.01
	RS	30	0.7554	0.7426	7.8018	0.017
	BA	31	0.7619	0.7484	7.6731	0.018
	MB	20	0.7567	0.7579	7.966	-0.002

Species	Site	n	H_E	H_O	A_R	F_{IS}
C. insignis						
	UEF	33	0.8134	0.8259	10.37033	-0.0160
	BC	9	0.8118	0.7778		
	WF	30	0.8041	0.7937	11.24289	0.013
	MF	32	0.8045	0.7920	11.73033	0.0160
	HB	30	0.8203	0.8109	10.99267	0.0120
	LC	31	0.7698	0.8290	7.896444	-0.0780
	LEF	30	0.8003	0.7986	10.54589	0.00
	GV	28	0.8181	0.8154	9.786111	0.00
	GF	7	0.8224	0.8810		
	RS	28	0.8038	0.8595	10.20967	-0.071
	BA	31	0.8296	0.8259	11.73722	0.004
	MB	3	0.8889	0.8889		
A. natalis						
	UEF	32	0.2738	0.3030	2.1506	-0.1090
	MF	37	0.3259	0.3844	2.2332	-0.1820
	HB	12	0.3277	0.3426	2.2222	-0.0480
	LEF	16	0.3392	0.3472	2.2699	-0.03
	GV	21	0.3124	0.2892	2.1887	0.08
	BA	4	0.2778	0.3333		
M. dolomieu						
	UEF	5	0.4713	0.3950		
	WF	2	0.3167	0.3500		
	MF	35	0.5233	0.5400	2.8889	-0.0330
	HB	26	0.4928	0.4494	2.7995	0.0900
	LEF	30	0.5133	0.5652	2.8978	-0.1030
	GV	29	0.5151	0.4989	3.0338	0.0320
	TC	2	0.5833	0.5500		
	GF	30	0.4973	0.4670	2.7672	0.06

Species	Site	n	H_E	H_O	A_R	F_{IS}
	RS	31	0.4388	0.3676	2.7489	0.17
	BA	5	0.3333	0.3200		

Table B.3. Estimates of local Ne of Gila River fishes sampled in 2010 using the program LDNE. Also included are sample sizes for the estimate (*n*), and lower and upper 95% confidence intervals (CI) estimated from LDNE. Many upper CI bounds included infinity (inf).

Species	Population	*n*	NeD	Lower 95% CI	Upper 95% CI
A. chrysogaster	WF	36	84.7	43.7	447.1
	MF	30	-248.7	254.4	inf
	HB	30	-853.1	172.3	inf
	BCA	30	-5880.0	101.2	inf
	GV	28	-1176.3	128.5	inf
	SAP	30	162.2	67.8	inf
	TURC	30	69.9	42.3	165
	GF	31	855.0	141.7	inf
	RS	30	-5212.1	126.4	inf
	BA	32	-399.6	326.1	inf
	MB	29	-1389.7	174.2	inf
	BLUC	30	-401.0	134.0	inf
	SSC	31	289.1	121.5	inf
M. fulgida	WF	30	252065.8	176.9	inf
	MF	34	212.5	82.3	inf
	HB	30	100.2	58.8	279.9
	RS	28	207.7	90.2	inf
	BA	31	4317.3	178.7	inf
	MB	17	-748.0	50.3	inf
R. osculus	UEF	19	-333.1	115.6	inf
	BCA	29	161.1	63.3	inf
	WF	26	208.7	68.3	inf
	MF	30	117.2	51.5	inf
	HB	30	150.6	60.1	inf
	LC	32	124.6	65.1	654.4
	GV	30	-197.1	314.7	inf
	BLUC	30	-3162.0	88.7	inf
T. cobitis	GV	30	140.6	70.7	1352.7
	RS	29	249.4	57.9	inf
	BA	34	597.7	149.6	inf
	MB	19	-239.9	349.3	inf
G. nigra	WF	26	52.8	27.9	210
	MF	20	61.5	27.1	inf
	UEF	19	58.4	28.5	518.2

Species	Population	*n*	NeD	Lower 95% CI	Upper 95% CI
	GV	12	8.2	5.9	11.4
C. insignis	WF	30	116.7	59.4	865.9
	MF	31	-605.2	209.3	inf
	HB	30	-240.8	297.1	inf
	LC	31	9.5	7.6	11.7
	UEF	33	6220.8	168.8	inf
	LEF	30	237.5	79.8	inf
	GV	28	193.1	81	inf
	RS	28	724.8	94.9	inf
	BA	31	343.1	78.4	inf
P. clarkii	WF	30	268.7	95.4	inf
	MF	32	299.8	70.1	inf
	HB	31	88.9	53.5	217.6
	LC	31	39.6	29.7	56.3
	UEF	32	227.2	90.5	inf
	BCA	35	50.3	34.4	84.1
	LEF	29	65.1	42.8	122.9
	GV	29	34.6	22.5	63.2
	GF	14	39.3	21.8	126
	RS	30	100.4	55.1	376
	BA	31	136.4	62.4	inf
	MB	20	422.8	72.7	inf
A. natalis	UEF	32	36.3	2.5	inf
	MF	37	16.8	3.7	127.1
	HB	12	100.4	2.1	inf
	LEF	16	42.8	5.3	inf
	GV	21	36.5	4.5	inf
M. dolomieu	MF	35	31.8	13.2	196.8
	HB	26	62.9	16.7	inf
	LEF	30	12.3	4.6	37.5
	GV	29	155.9	18.2	inf
	GF	30	21.1	8.4	122.4
	RS	31	23.8	6.7	inf