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RESEARCH ARTICLE

Comparative conservation genetics of protected endemic fishes in an arid-land riverscape

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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 ($N_e \sim 100$) and temporally unstable population structure. *Meda fulgida* and *R. 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.

Keywords Multispecies management · Gene flow · Evolutionary potential · Effective population size · Landscape genetics · Microsatellites

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

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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 *Rhinichthys* (*Tiaroga*) *cobitis* (herein referred to as *Tiaroga cobitis* for consistency with previous published research of this species). 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. 2015); 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) (Fig. 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 the Aqua Fria River,

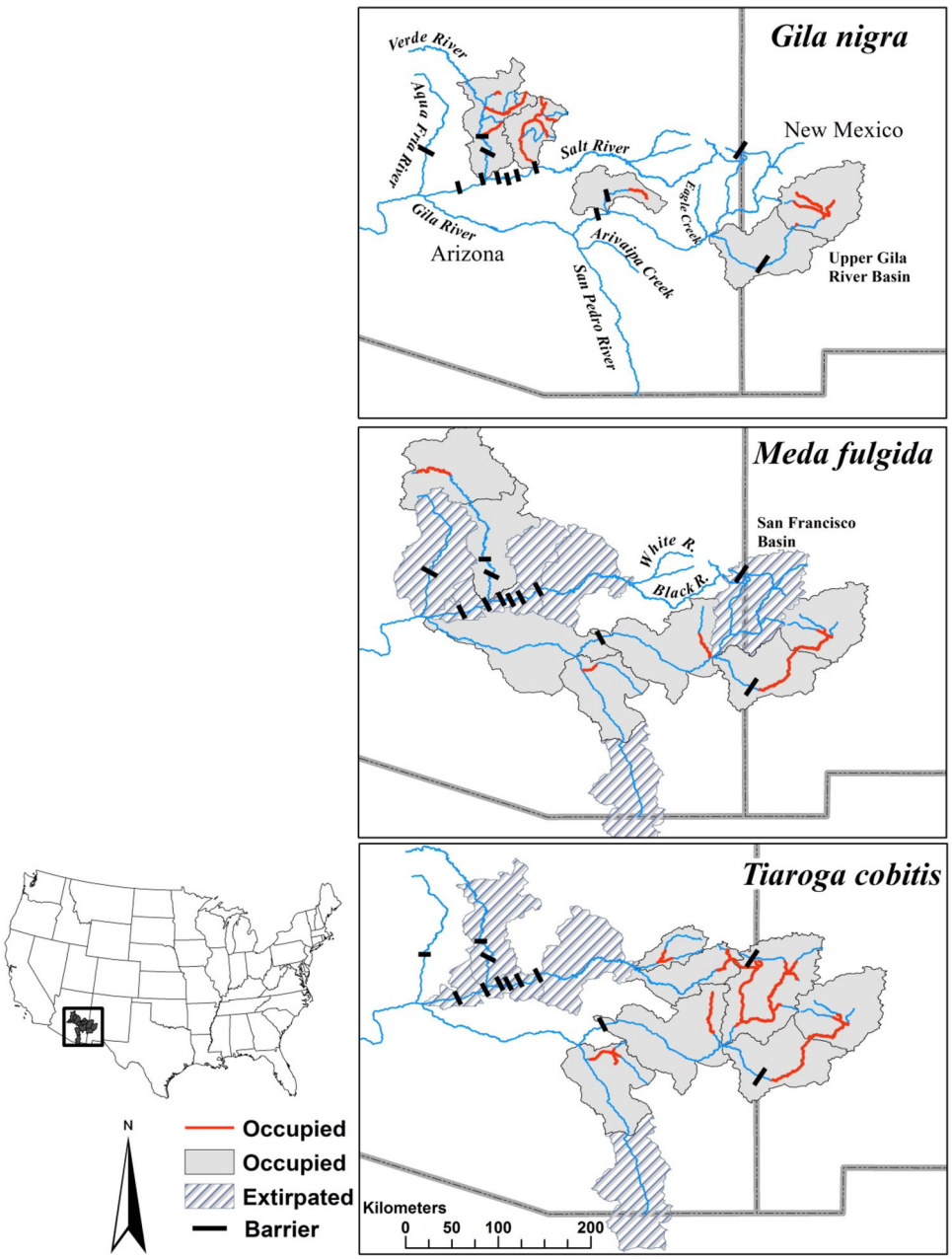

Fig. 1 Range maps for three protected fishes endemic to the Gila River basin of New Mexico and Arizona, USA, indicating historical and current distributions in 8-digit USGS hydrologic unit code (HUC) watersheds. Streams currently occupied are indicated in *red*. Data on current and historical distributions from NatureServe (www.natureserve.org) and U.S. Bureau of Reclamation (http://www.usbr.gov/lc/phoenix/biology/azfish/profintro.html). (Color figure online)

including the Verde River, Salt River, and San Pedro River catchments of southeastern and central Arizona and southwestern New Mexico (Fig. 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 Forest Wilderness Area (Fig. 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 (1161–2059 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 × 1.2 m, 3.2 mm 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).

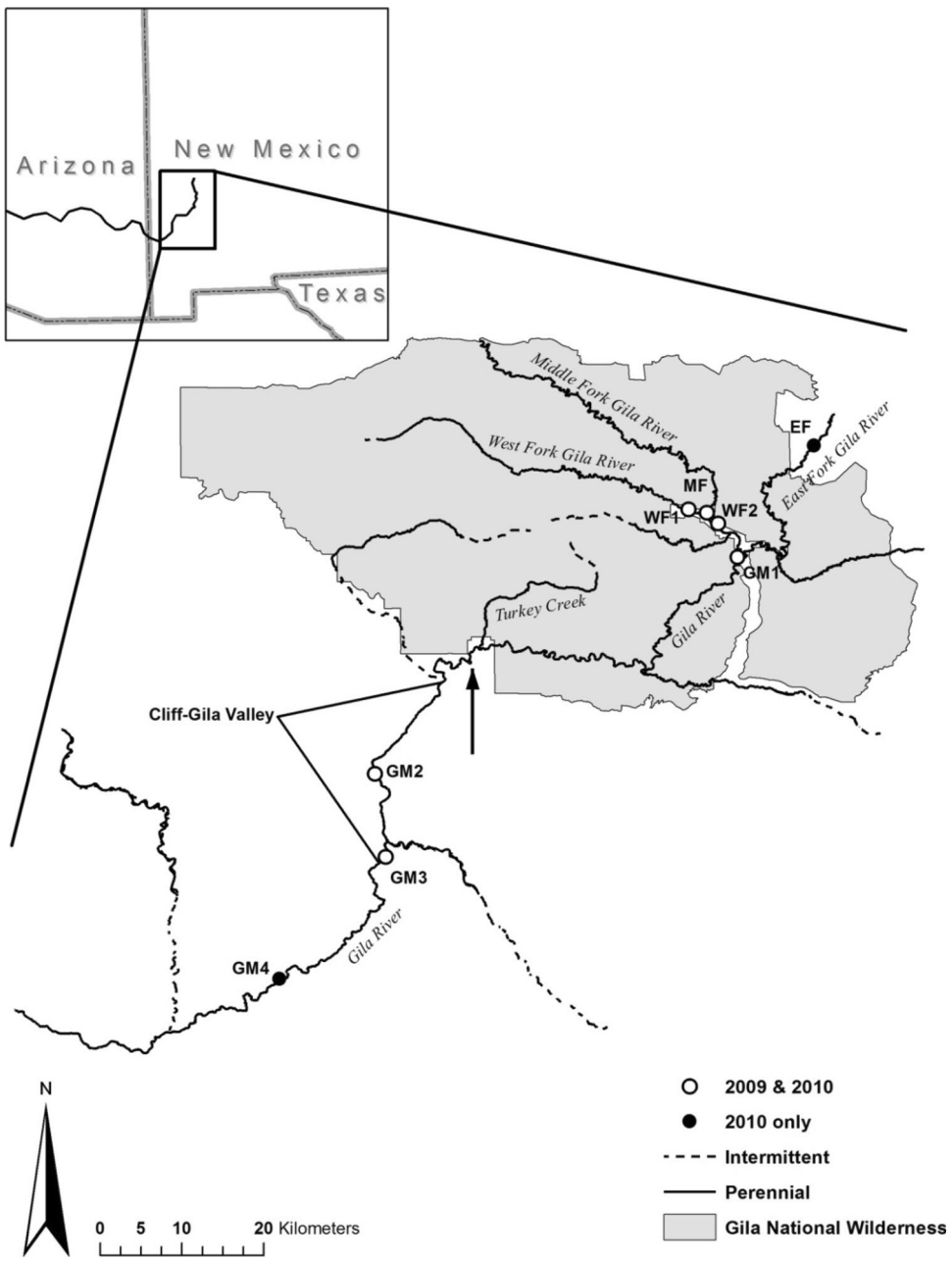

Fig. 2 Sample sites for three protected fishes of the upper Gila River catchment, New Mexico, USA. Samples collected from locations in 2009 are indicated with *open circles* and additional locations sampled in 2010 indicated by *closed circles*. *Large arrow* represents the approximate location of a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

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 A1, Electronic Supplementary Material). 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 re-analyzed 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). *Gila nigra* (n = 149) was 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). 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–GM4) (Table 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 A1, Electronic Supplementary Material). *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 1 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.

Table 1 Genetic summary statistics for three protected fishes of the upper Gila River catchment sampled in 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

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

Intra-specific genetic diversity and effective size

Mean observed heterozygosity (H_O) for *G. nigra* across sites and years was 0.63 (range 0.60–0.66), 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) 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). 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 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 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 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 A2, Electronic Supplementary Material). 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* ($r = 0.98$, $P = 0.037$; Fig. 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 (Fig. 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* ($A_R = 6.6$–6.4) and *T. cobitis* ($A_R = 10.4$–10.5). The greatest degree of temporal

Table 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

Species	N_{eD} (95 % CI)		N_{eS} (95 % CI)	
	2009	2010	2009	2010
Gila nigra	80 (61–112)	105 (78–151)	60 (41–89)	83 (6–117)
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 3 Population level F_{ST} values for three protected fishes of the upper Gila River catchment sampled during 2009 and 2010

Species	2009 F_{ST} (95 % CI)	2010 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)

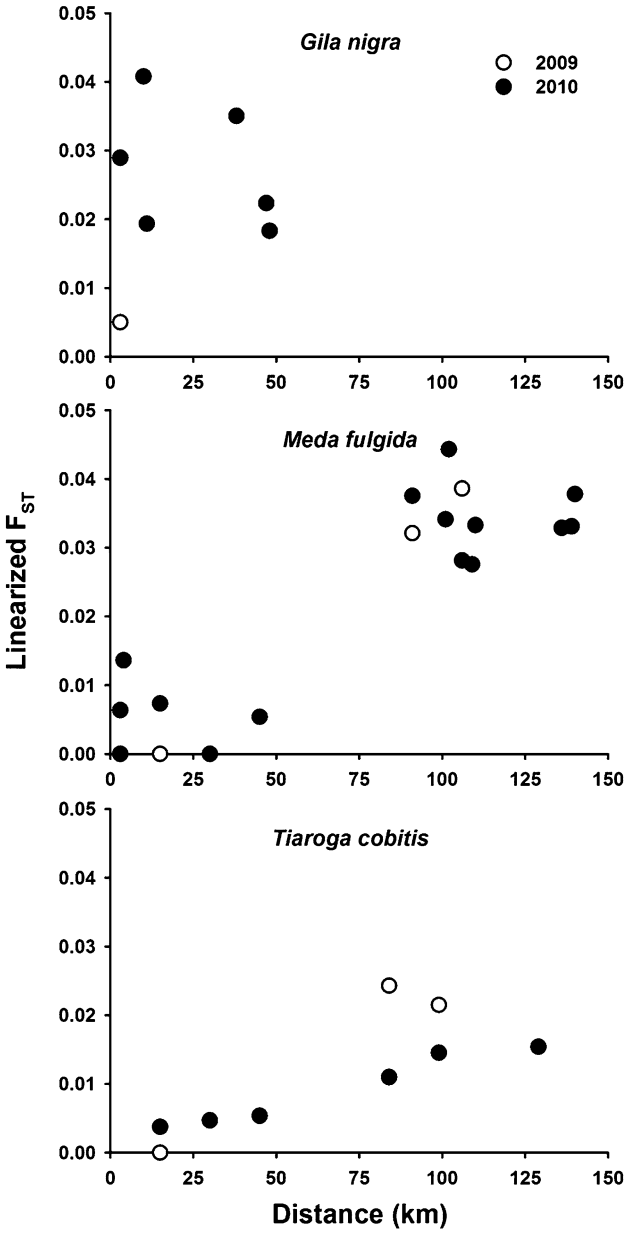

Fig. 3 Biplots of pairwise linearized F_{ST} versus stream distance in kilometers for three protected fishes of the upper Gila River catchment, New Mexico, USA. Samples collected in 2009 indicated by *open circles* and 2010 by *closed circles*

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). Genetic effective size was consistent between years for *G. nigra* because the 95 % CIs overlapped (Table 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 3; Fig. 3). 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 (Fig. 3).

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

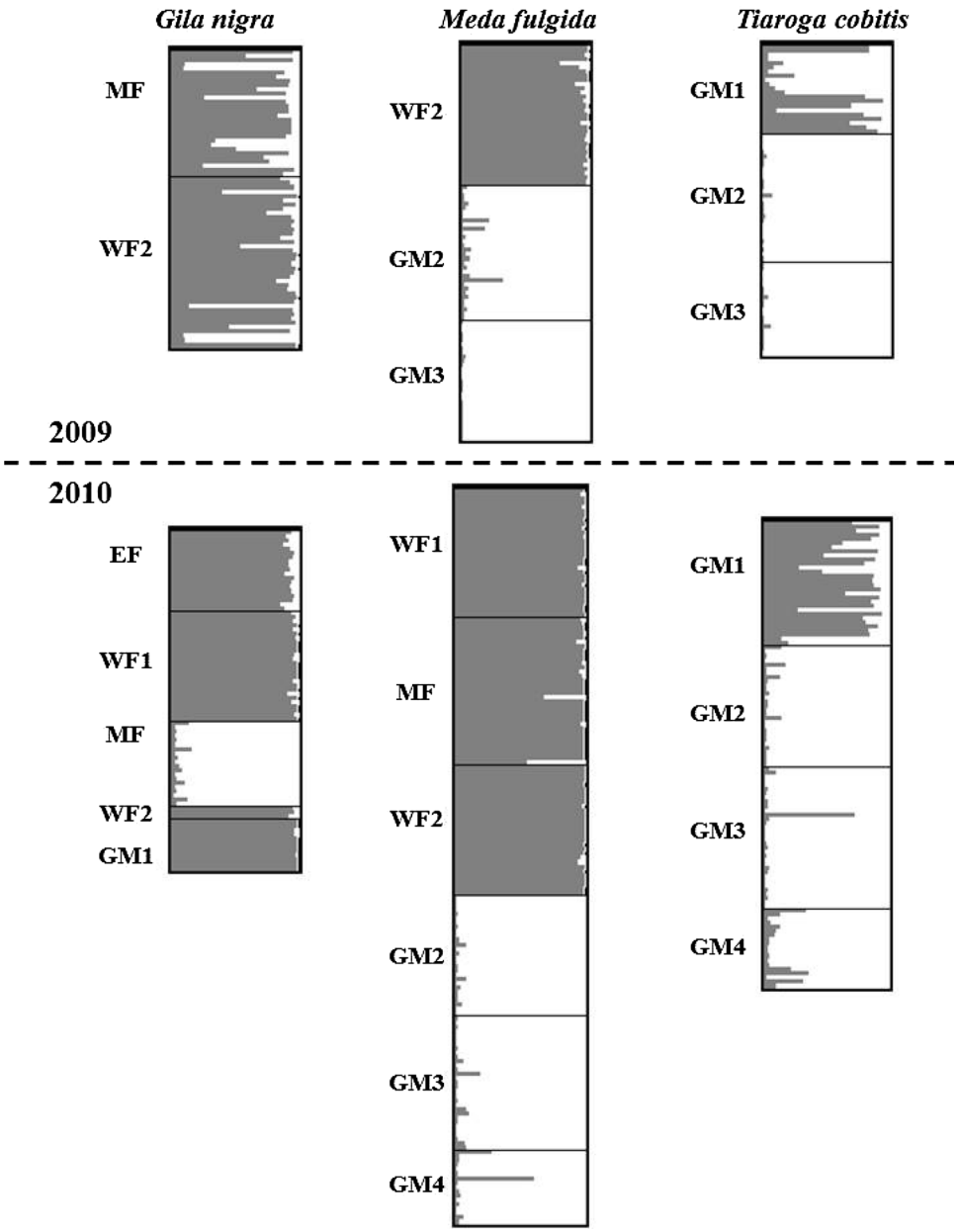

Fig. 4 Assignment probability plots obtained from STRUCTURE for three protected fishes of the upper Gila River catchment sampled in 2009 (above *dashed line*) and 2010 (*below*). Each *horizontal bar* represents an individual and the probability of being assigned to one of two genetic units (K = 2, represented by either *gray* or *white*). Site abbreviations correspond to Table 1 and Fig. 2

(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 et 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; Figs. 3, 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 60–600 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. 2015). 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).

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