

Genetic characterization and management of the endangered Mohave tui chub

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Abstract The Mohave tui chub (*Siphateles bicolor mohavensis*) is the only fish native to the Mojave River, California. The fish were displaced by introduced arroyo chubs (*Gila orcutti*) throughout most of their range, starting in the 1930s. Two potentially relictual populations and two transplanted populations were genetically characterized using 12 microsatellite DNA loci, along with contemporary cyprinid populations in the Mojave River. We found only un-hybridized Mohave tui chubs in the refuge populations, and only un-hybridized arroyo chubs in the Mojave River. The two largest Mohave tui chub populations (Lake Tuendae and China Lake) exhibit similar, comparatively high genetic variation. Another large population (Camp Cady) with low genetic diversity shows the effect of a bottleneck of ten individuals during the historic founding event. The fourth population (MC Spring) has the fewest alleles, lowest heterozygosity, and is the most divergent, suggesting that genetic drift from a persistently low effective population size has reduced genetic diversity

since its apparent isolation in 1934. We recommend instituting artificial gene flow to rebuild genetic variation in Camp Cady from both Lake Tuendae and China Lake, and the establishment of new populations with founders from both Lake Tuendae and China Lake. Additionally, we comment on the infeasibility of restoring populations of Mohave tui chub in their historic habitats.

Keywords Tui chub · Microsatellite DNA · Translocation · Mojave River · Hybridization · Conservation

Introduction

Tui chubs (*Siphateles bicolor* Girard 1856) are long-lived, omnivorous Cyprinid fishes widely distributed among the interior basins of western North America (Sigler and Sigler 1987; Crain and Corcoran 2000; Moyle 2002). Distinctive forms have arisen in response to isolation in geologic basins (Hubbs and Miller 1948; Harris 2001; Chen et al. 2007, 2009). However, due to habitat degradation and nonnative fish invasions, a number of tui chub subspecies have declined and/or have become extinct (Williams et al. 1989; Moyle and Williams 1990). The southernmost subspecies *S. b. mohavensis* (Snyder 1918) is endemic to the Mojave River, a closed drainage basin in southern California, where it is the only native fish (Snyder 1918; Hubbs and Miller 1948). Natural Mohave tui chub populations were formerly widespread within the Mojave River in historical times (Uyeno and Miller 1963; Grayson 1993), occurring as far downstream as Soda Lake when sufficient water flowed from the San Bernardino Mountain headwaters. A history of anthropogenic extirpation, population fragmentation, partial resurgence under purposeful

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management, and concern for genetic consequences is shared by a growing number of aquatic and terrestrial animals. Diverse examples in addition to the Mohave tui chub include: Northern Leopard Seal (Bonnell and Selander 1974), Sierra Nevada Bighorn Sheep (Johnson et al. 2011), and Kirtland's Warbler (Wilson et al. 2012).

Mohave tui chubs have been eliminated from their natural range, following introduction of arroyo chubs (*Gila orcutti* Eigenmann and Eigenmann 1890). Arroyo chubs originate from drainages of the Los Angeles coastal plain, separated from the Mojave River by the 3,000+ m high San Bernardino Mountains. Presumably, arroyo chubs were introduced as fishing bait in tributary streams, where they remain established above dams and waterfalls, and from there spread downstream into the historic Mohave tui chub habitat. During the 1930s, Hubbs and Miller (1943) documented an increase in arroyo chubs, hybridization between arroyo chubs and Mohave tui chubs, and a rapid decline of the latter species in the Mojave River. Ultimately the species' range became restricted to two small isolated fragments of its former habitats.

In 1970, the United States Fish and Wildlife Service (USFWS) listed the Mohave tui chub as endangered under the federal Endangered Species Act (FR 16047 16048). The following year, the subspecies was added to the state of California's list of endangered species. In 1984 a "Recovery Plan for the Mohave tui chub" (Plan) was prepared by the USFWS. The Plan foresees two potential levels of recovery for the species. First, the minimum number of populations (which meet certain criteria) will be increased to six, at which time the subspecies may be considered for reclassification from "endangered" to "threatened." Second, the Plan provides for active management of the refuge populations, including interchange and mixing of individuals to avoid loss of genetic diversity through inbreeding. To be considered for eventual removal from the endangered species list, viable populations of Mohave tui chub need to be successfully established "in a majority of the species' historic habitat." This larger goal may not be accomplished if the arroyo chub is still present throughout the Mojave River and its tributaries.

Mohave tui chubs and arroyo chubs cannot be reliably distinguished in hand without dissection and microscopy, despite their considerable taxonomic separation. Scientists and managers considered formation of a hybrid swarm to be responsible for elimination of Mohave tui chubs from their native range (Miller 1969; U.S. Fish and Wildlife Service 1984). However, May (1997) reported the results of an allozyme and AFLP (amplified fragment length polymorphism) study that unexpectedly did not detect any genetic contribution by Mohave tui chubs in the putative

hybrids. At the outset of our study the identity and interactions of cyprinid fishes in the Mojave River were poorly understood.

Mohave tui chubs retain anatomical and physiological traits consistent with their evolutionary heritage as lake-dwelling fish during the Pleistocene (Hubbs and Miller 1943; Uyeno and Miller 1963; Grayson 1993). In contrast, arroyo chubs are stream-adapted minnows. A comparative physiological study indicates that arroyo chubs are stronger swimmers, and more tolerant of low dissolved oxygen and warm temperatures than are Mohave tui chubs (Castleberry and Cech 1986). Anatomic comparisons show the arroyo chubs to be better adapted for stream life than Mohave tui chubs, based upon coloration, gill raker structure, dentition, and body shape (Hubbs and Miller 1943). It is possible that competitive displacement by arroyo chubs was sufficient to extirpate Mohave tui chubs, whether or not genetic interactions played a role. Regardless of the mechanism, Mohave tui chubs no longer exist in the Mojave River; they persist only in isolated or artificial refuges.

Extant Mohave tui chubs persist in two potentially relictual populations, Lake Tuendae and Mohave Chub Spring (MC Spring), located 260 m apart on the shore of Soda Dry Lake in Mojave National Preserve (Miller 1938). There is no evidence of either natural or artificial interchange of individuals between these populations in historic times. Experimental translocations made from Lake Tuendae into 13 new habitats in the 1970s (Hoover and St. Amant 1983) resulted in one long-term success, the establishment of Mohave tui chub at China Lake Naval Air Weapons Station (China Lake). Subsequently, tui chubs from Lake Tuendae were established in artificial habitats at Camp Cady State Wildlife Area (Camp Cady) in 1986. Currently, all four populations are in artificial, or highly modified, habitats. Unpublished population studies indicate Lake Tuendae, Camp Cady, and China Lake each number in the thousands of individuals and are demographically robust. In contrast, the MC Spring population consists of 250–600 individuals characterized by small body size (authors' unpublished data) and limited by its restricted habitat area.

The establishment of new populations by translocation entails a risk of losing genetic variability and/or increasing inbreeding in the daughter population(s) (Conant 1988; Stockwell et al. 1996; Storfer 1999; Mock et al. 2004). Insights from genetic studies allow managers to understand the past and present genetic status of populations, to improve the genetic health of populations, to discern unrecognized management needs, and to devise new management strategies to promote long-term population viability (Chesser 1983; Hedrick 1983; Echelle 1991;

Chen et al. 2011). Prior to this study, the only genetic data on Mohave tui chubs were based on allozyme and AFLP (May et al. 1997), and DNA sequencing (Harris 2001). These studies demonstrate the Mohave tui chub to be genetically distinct from other tui chubs, including the Owens (*S. b. snyderi*), Lahontan (*S. b. obesa* and *S. b. pectinifer*), and Klamath Lake (*S. b. bicolor*) subspecies, but provide little insight into within-subspecies variability. The markers used in prior studies lack sufficient resolution to characterize genetic variation within and among the four Mohave tui chub populations.

Microsatellite DNA loci (microsatellites) are hyper-variable, suitable for population genetics and hybridization studies (Tautz 1989; Weber and May 1989; Roy et al. 1994). This study complements previous genetic work on this species by using microsatellites and larger sample sizes to (1) assess historical hybridization between the Mohave tui chub and arroyo chub; (2) analyze the population structure and genetic variation within and among Mohave tui chub populations; (3) evaluate whether any of the populations have undergone detectable bottleneck; (4) identify appropriate stocks and genetic strategies for reestablishment of the Mohave tui chub; (5) recommend population-specific actions for long-term and possible downlisting/delisting of Mohave tui chub.

Fig. 1 Map shows the sample collection sites of Mohave tui chubs in Camp Cady, China Lake, Lake Tuendae, and MC Spring (labeled with *green squares*) and arroyo chubs in Afton Canyon, Upper Narrows, and San Margarita River (labeled with *yellow squares*). (Color figure online)



Materials and methods

Sample collection

Fish were captured with funnel traps to represent three groups: (1) Mohave tui chubs in refuges, (2) contemporary Mojave River cyprinids of uncertain ancestry (putative hybrid swarm), and (3) known arroyo chubs. A single pelvic fin tip was removed for analysis, and all Mohave tui chubs were released at the point of capture. Fin tissue (10–50 mm²) was placed in a paper envelope, air-dried, and stored at room temperature until needed. Mohave tui chub collections were authorized under federal fish and wildlife permit TE161225-0.

Forty-eight individual Mohave tui chubs were collected in 2005 representing each of the four refuge populations: Camp Cady (34°56'12"N, 116°36'42"W), China Lake (35°42'00"N, 117°37'48"W), Lake Tuendae (35°08'36"N, 116°06'15"W), and MC Spring (35°08'27"N, 116°06'15"W). Unidentified Mojave River cyprinids were represented by 48 specimens collected in Afton Canyon (35°02'16"N, 116°22'52"W) in 2005, and eight individuals from the "Upper Narrows" of the Mojave River at Victorville, San Bernardino County (34°34'26"N, 117°19'21"W) collected in 1997 (Fig. 1). Arroyo chubs within their native

range were represented by four specimens from the Santa Margarita River, San Diego County (33°19'23"N, 117°09'30"W) collected in 1997.

DNA extraction, PCR amplification and microsatellite genotyping

Whole genomic DNA was extracted from tissue samples using the Promega 96-well Tissue Kit (Promega Corporation).

Twelve selected microsatellite loci employed in this study. Of these, eight (Gbi-G3, Gbi-G10, Gbi-G13, Gbi-G27, Gbi-G38, Gbi-G39, Gbi-G79, and Gbi-G87) were developed by Meredith and May (2002), and four (CypG3, CypG41, CypG47, and CypG48) were from Baerwald and May (2004). The forward primer of each primer pair was labeled with a fluorescent phosphoramidite (FAM, TET, VIC, HEX, or NED; Applied Biosystems). Microsatellite DNA was amplified via polymerase chain reaction (PCR). Each 10 μ L PCR contained: 20 mM Tris–HCl, pH 8.4, 1.5 mM MgCl₂ (3.0 mM for Gbi-G13 and Gbi-G38), 0.2 mM dNTPs, 0.5 μ M unlabeled reverse primers, 0.025 μ M labeled forward primers, and 0.4 units Taq DNA polymerase (Promega). Reaction mixtures were amplified using the following conditions: 96 °C for 2 min, followed by 35 cycles of 95 °C for 40 s, 52 °C for 1 min, and 72 °C for 1 min, ending with an extension of 72 °C for 10 min. The PCR-generated microsatellite DNA products were visualized on a BaseStation gel imaging system (BioRad). Composite genotypes for individual fish were compiled by scoring co-dominant alleles at each microsatellite locus using Cartographer 1.2.6 software (BioRad).

Population genetic data analyses

We used CONVERT 1.2 (Glaubitz 2004) to compute allelic frequencies, and to score the number of private alleles for each locus and population. We used FSTAT 2.9.3 (Goudet 1995) to score the total number of alleles and allele richness; the latter predicts the number of alleles independent of sample size (El Mousadik and Petit 1996) averaged over loci for each population. We employed MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to identify null alleles and other genotyping errors of the dataset, and ARLEQUIN 3.0 (Excoffier et al. 2005) to test for deviations from Hardy–Weinberg equilibrium using 10,000 Markov chain and 1,000 dememorization steps, and to compute observed heterozygosity (H_O) and expected heterozygosity (H_E).

We used LDNE 1.31 (Waples and Do 2008) to estimate the effective population size (N_e) assuming random mating within populations. All alleles with frequencies less than 0.01 were excluded in the estimation. We used GENETIX 4.04 (Belkhir et al. 2003) to perform pairwise values for

Weir and Cockerham's (1984) F_{ST} and Cavalli-Sforza and Edwards (1967) chord's distance (D_{CE}) and their statistical significance tests to visualize genetic similarities among different populations. The statistical significance of pairwise F_{ST} and D_{CE} values was tested via 10,000 permutations and a standard Bonferroni correction.

Population structure for Mohave tui chubs was modeled using STRUCTURE 2.1 (Pritchard et al. 2000), a Bayesian clustering method that can assume admixture and correlated allele frequencies between K number of clusters. Iterations included a 100,000 burn-in period, 100,000 Markov chain Monte Carlo reps after burn-in, and an admixture model with an initial value of $\alpha = 1.0$ (Dirichlet Parameter for Degree of Admixture) and a maximum value = 10.0. Allele frequencies were correlated among populations with a constant value of $\lambda = 1.0$ (Allele Frequencies Parameter); no prior information on individual sample localities was taken into account. Models were run at $K = 1–6$ for ten replicates at each K to verify consistency of untransformed log-likelihood probabilities, $L(K)$. A consistent maximum value for $L(K)$ was interpreted to reflect the true population structure (Pritchard et al. 2000; Falush et al. 2003). We used an ad hoc criterion of ΔK derived from Evanno et al. (2005) in addition to $L(K)$, to find the best number of clusters (K).

We evaluated recent population bottlenecks using BOTTLENECK 1.2.02 (Piry et al. 1999). In this test we implemented a two-phase model (TPM) and Wilcoxon's two-tailed significance test for heterozygosity excess, or deficiency, for 10,000 replications. TPM is thought to be superior for microsatellite data to either IAM or SMM (Piry et al. 1999). Wilcoxon's test is believed to be the most powerful and robust test for data with fewer than 20 loci (Piry et al. 1999).

Results

Inter-specific diagnostic variation

For each of twelve microsatellite DNA loci screened, all 56 putative hybrids from the Mojave River (48 from Afton Canyon and eight from Upper Narrows) are similar to arroyo chubs and distinct from Mohave tui chubs. Putative hybrids from the Mojave River and arroyo chubs are each characterized by poor amplification at six loci (Gbi-G10, Gbi-G27, Gbi-G39, Gbi-G79, Gbi-G87, and CypG3); whereas refuge Mohave tui chub samples all successfully amplified (see Appendix 1 in supplementary material). Four loci (Gbi-G10, Gbi-G27, Gbi-G79, and Gbi-G87) show no amplification in the Mojave River "hybrid" and arroyo chub samples, and two additional loci (Gbi-G39 and CypG3) amplified in fewer than 10 % of individuals.

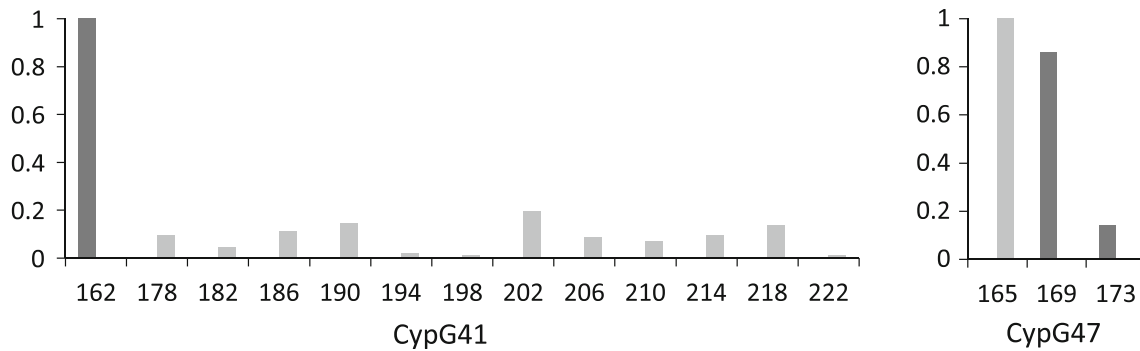


Fig. 2 Allelic frequency and distribution for Mohave tui chubs (*Dark gray bar*) from China Lake, Lake Tuendae, MC Spring and Camp Cady, and for arroyo chubs (*Light gray bar*) from Afton Canyon,

Victorville and San Margarita River at two microsatellite loci, CypG41 and CypG47 possessing diagnostic alleles. X-axis represents the frequency of alleles, and Y-axis represents the size of alleles

At the remaining six loci, the Mojave River and arroyo chub samples share the majority of alleles, for which the allele range is 340–396 bp (Gbi-G3), 212–272 bp (Gbi-G13), 354–466 bp (Gbi-G38), 178–222 bp (CypG41), 165 bp (CypG47), and 221–273 bp (CypG48). The allele range for individual loci Gbi-G3, Gbi-G13, Gbi-G38, CypG41, CypG47, and CypG48 does not overlap between Mohave tui chub and either putative hybrid or arroyo chub samples. In locus Gbi-G13 the size range overlaps among the three groups, but no alleles are shared between Mohave tui chubs and either Mojave River samples or arroyo chubs. Two diagnostic alleles occur at CypG41 and CypG47 which distinguish all Mojave River samples and arroyo chubs from Mohave tui chubs. The first diagnostic allele is 162 at locus CypG41, which is fixed in Mohave tui chubs. The second diagnostic allele, 165 at CypG47, is fixed in arroyo chubs and the putative hybrids, while Mohave tui chubs display two alternate alleles, 169 and 173 (Fig. 2).

Microsatellite DNA variation in Mohave tui chubs

Eleven of 12 microsatellite loci are polymorphic, while CypG41 is monomorphic for allele 162 in the Mohave tui chub. Locus CypG41 was dropped from all of the following analyses due to its invariance. The allele frequencies and number of alleles (N_A) were computed for each population at each locus (Appendix 1 in supplementary material). The polymorphic loci had between two (CypG47) and ten (Gbi-G27) alleles for Mohave tui chubs. Ten loci are tetranucleotide repeats, while one locus (Gbi-G79) is irregular. The same irregular repeat pattern at Gbi-G79 was previously reported in Lahontan tui chubs (Chen et al. 2007) and is due to a 1 bp indel named “Sb-D” in the flanking region of Gbi-G79. Microsatellites from three Mohave tui chubs homozygous at Gbi-G79 were

sequenced to confirm the indel in this species. The frequencies of Sb-D vary among the four Mohave tui chub populations, ranging from 0.25 at Camp Cady, 0.39 at Lake Tuendae, 0.43 at China Lake, and 0.80 at MC Spring (Table 1).

Two of 43 tests indicate deviation from Hardy–Weinberg equilibrium at locus Gbi-G79 in China Lake and MC Spring ($p \leq 0.01$) (Appendix 1 in supplementary material); however, MICRO-CHECKER tests show no evidence for null alleles at Gbi-G79 ($p > 0.01$), so this locus was included in the analysis.

Of the four Mohave tui chub refuge populations, MC Spring possesses fewer alleles and less allelic richness (average $N_A = 3.09$; $R_A = 3.06$) than either Camp Cady (average $N_A = 3.73$; $R_A = 3.69$), China Lake (average

Table 1 The number of alleles (N_A), allele richness (R_A), expected heterozygosity (H_E), and number of private alleles (N_P) averaged over 11 polymorphic microsatellite DNA loci, followed by the effective population size (N_e), frequency of Sb-D at Gbi-G79 (P_{Sb-D}), and p value of Wilcoxon’s test ($P_{WILCOXON}$)

	Camp Cady	China Lake	Lake Tuendae	MC Spring
N_A	3.73*	5.64	5.18	3.09*
R_A	3.69*	5.56	5.12	3.06*
H_E	0.53	0.60	0.58	0.40
N_P	0	0.55	0.36	0.09
N_e	231.5	∞	1974.5	211.5
P_{Sb-D}	0.25	0.43	0.39	0.80
$P_{WILCOXON}$	0.01*	1.00	0.49	0.21

Asterisks (*) indicate values observed in Camp Cady and MC Spring that are significantly lower than those in China Lake and Lake Tuendae ($\alpha = 1 \%$; two-tailed t test), and p value for Wilcoxon’s test that is significant ($p \leq 0.01$). ∞ represents that the estimate of N_e is infinity, indicating there is no evidence of linkage disequilibrium resulting from genetic drift due to a finite number of breeders

Table 2 Pairwise values for Weir and Cockerham's (1984) F_{ST} above diagonal and Cavalli-Sforza and Edwards (1967) chord's distance (D_{CE}) below diagonal among the four Mohave tui chub populations

Population	Camp Cady	China lake	Lake Tuendae	MC Spring
Camp Cady	–	0.07*	0.10*	0.26*
China Lake	0.08*	–	0.02*	0.17*
Lake Tuendae	0.08*	0.04*	–	0.17*
MC Spring	0.19*	0.13*	0.14*	–

Asterisks (*) indicate values of statistical significance ($p \leq 0.01$) following 10,000 permutations and a standard Bonferroni correction

$N_A = 5.64$; $R_A = 5.56$) or Lake Tuendae (average $N_A = 5.18$; $R_A = 5.12$). Expected heterozygosity (H_E) averaged over 11 polymorphic microsatellites at MC Spring ($H_E = 0.40$) is likewise exceeded by Camp Cady ($H_E = 0.53$), China Lake ($H_E = 0.60$), and Lake Tuendae ($H_E = 0.58$). MC Spring also has the lowest effective size ($N_e = 212$). Camp Cady has a small effective size ($N_e = 232$) compared with Lake Tuendae ($N_e = 1,975$) and China Lake ($N_e = \text{Infinity}$). Private alleles occur in three of the four populations. China Lake has the most average private alleles per locus, $N_p = 0.55$, Lake Tuendae follows with $N_p = 0.36$ and MC Spring had $N_p = 0.09$ (see Table 1). Some alleles that are common in MC Spring are rare in the other populations. For example, allele 187 at Gbi-G39 has a frequency of 0.63 at MC Spring, in contrast to frequencies of 0.04, 0.06 and 0.07 at Camp Cady, Lake Tuendae, and China Lake, respectively (Appendix 1 in supplementary material).

All pairwise values of Weir and Cockerham's F_{ST} and Cavalli-Sforza and Edwards chord's distance (D_{CE}) among Mohave tui chub populations are statistically significant ($p \leq 0.01$). MC Spring is the most divergent of the four populations, with values of $F_{ST} = 0.26$ and $D_{CE} = 0.19$ between MC Spring and Camp Cady, $F_{ST} = 0.17$ and $D_{CE} = 0.13$ between MC Spring and China Lake, and

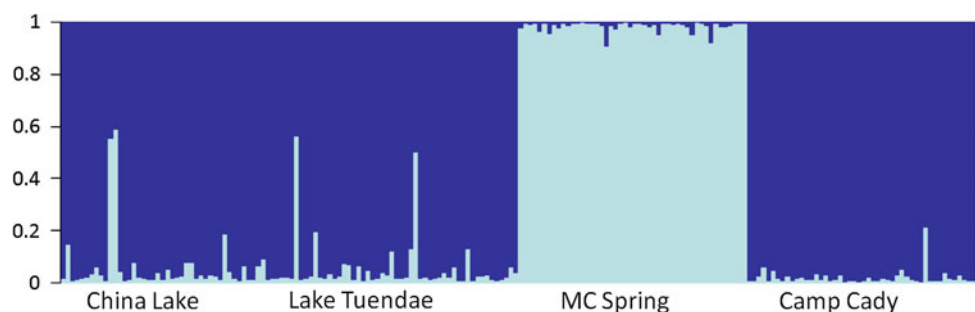
$F_{ST} = 0.17$ and $D_{CE} = 0.14$ between MC Spring and Lake Tuendae (Table 2). Camp Cady is the next most divergent population. Values of $F_{ST} = 0.07$ and $D_{CE} = 0.08$ between Camp Cady and China Lake, and $F_{ST} = 0.10$ and $D_{CE} = 0.08$ between Camp Cady and Lake Tuendae, are larger than values between China Lake and Lake Tuendae ($F_{ST} = 0.02$ and $D_{CE} = 0.04$).

Population substructure and demographic history of refuge populations

STRUCTURE and BOTTLENECK tests for Mohave tui chubs included all eleven polymorphic loci. Bayesian analysis using STRUCTURE shows log-likelihood probabilities increased with increasing K from 1 (−4418.27) to 2 (−4096.13), and 3 (−3955.87) and decreased with increasing K to 4 (−4028.87), 5 (−4065.70), and 6 (−4108.20), however the ad hoc value of ΔK (Evanno et al. 2005) was maximized for K = 2 (128.49) and then progressively declined by increasing K from 2 to 3 (89.38), 4 (6.52), and 5 (0.45). We justified an assignment of two *posteriori* clusters, specifically (1) MC Spring and (2) Camp Cady/China Lake/Lake Tuendae (Fig. 3). The MC Spring population formed the first cluster independently, indicative of its greatest difference from other Mohave tui chub populations; the majority of Camp Cady, China Lake, and Lake Tuendae individuals are characterized by a second cluster, with little difference apparent among the populations.

Results from BOTTLENECK suggest that that Camp Cady underwent a recent population bottleneck. All four Mohave tui chub populations exhibited significant excess heterozygosity from the infinite allele model (IAM), but no excess when using the step-wise mutation model (SMM). Assuming that the proportion of SMM is 70 % (probability = 0.70), the TPM test at 10,000 replications for Camp Cady indicates excess heterozygosity ($p = 0.01$), signifying a recent population bottleneck. The remaining three Mohave tui chub populations do not significantly depart from mutation/drift equilibrium (Table 1).

Fig. 3 Bayesian clustering of Mohave tui chubs using STRUCTURE analysis suggests an assignment of two *posteriori* clusters ($K = 2$). The value on the vertical axis is the probability that each individual is assigned to a specific population



Discussion

Historical introgression between the Mohave tui chub and arroyo chub

Our results using microsatellites, larger sample sizes, and an additional collection locality at Afton Canyon increase the certainty and support the findings of May et al. (1997) that there is no evidence of Mohave tui chub ancestry within the extant Mojave River cyprinids. The abundant cyprinids in the Mojave River are arroyo chubs (*Gila orcutti*), notwithstanding Hubbs and Miller's (1943) observations of mass hybridization throughout the Mojave River. While hybridization may have occurred historically, the absence of hybrids today suggests reduced fitness, or infertility, may occur in F1 or subsequent introgressed progeny (e.g. F2 and backcross). In light of the fact that introgression was not responsible for the demise of the Mohave tui chub, we attribute their extirpation to competition with, and potential reproductive interference by, arroyo chubs—a species which is ironically better suited to contemporary conditions in the Mojave River.

Mohave tui chubs evolved as a pluvial lake-dwelling fish (Grayson 1993). Holocene climate change eliminated the lakes, naturally restricting the fish to less favorable low elevation habitats with seasonally very warm water. However, upstream of waterfall barriers to fish colonization, cool tributary streams with deep bedrock pools exist and are populated by introduced arroyo chubs. Since hybridization has not resulted in introgression, managers should reconsider the potential conservation significance of upstream habitats which were previously dismissed because of the presence of arroyo chubs. Conceivably, higher elevation tributary streams and lakes of the Mojave River watershed may favor Mohave tui chubs to the extent they may coexist with, or even out compete, arroyo chubs.

Our diagnostic markers provide a necessary non-lethal tool for distinguishing Mohave tui chubs, arroyos chubs and their hybrids. This capacity will enable novel experiments to explore coexistence and competition between these two species, facilitate evaluation of recovery projects, and create hope for reestablishing Mohave tui chub populations in nature.

Genetic variation and population substructure of Mohave tui chub

The Mohave tui chub is the southernmost of ten or more subspecies within the tui chub complex (Harris 2001; Moyle 2002). Tui chubs are thought to have derived from the pluvial Lahontan basin, and spread into other drainages via Pleistocene water connections. With the retreat of pluvial water, ancestral Mohave tui chub were isolated

(Hubbs and Miller 1948; Soltz and Naiman 1978; Jayko et al. 2008). Mohave tui chubs were widespread in the historic habitat before arroyo chubs were introduced. Isolation into refuge populations has apparently diminished the genetic variability of some or all extant populations.

Evidently, significant genetic changes have occurred in some Mohave tui chub populations since the 1930s. Lake Tuendae is the source of the founders which established populations at China Lake in 1971 and at Camp Cady in 1986. Three annual population estimates for Lake Tuendae Mohave tui chub vary between 2,241 and 3,708 (Garron 2006), and the China Lake population is probably larger based upon known occupied habitat area. The estimates of effective population size reinforce the hypothesis that China Lake supports a larger census population size than Lake Tuendae. In addition, little divergence between Lake Tuendae and China Lake suggests that neither population has undergone a significant bottleneck since the 1971 founding event of China Lake. Interestingly, there are private alleles present in China Lake which are absent from Lake Tuendae, the source population. This may be explained by sampling error, or loss of rare alleles due to genetic drift in the smaller Lake Tuendae. Hughson and Woo (2004) document human-caused disturbances that may have temporarily reduced population numbers in Lake Tuendae, suggesting a possible history of minor bottleneck(s).

Camp Cady also has much lower genetic diversity compared with the source population, Lake Tuendae, although survey data indicate similar census sizes for both populations. Agency records show Camp Cady was populated with an initial ten fish released in the summer of 1986, followed by an augmentation of 55 in the following year. Vicker (1973) found Mohave tui chub females contain 3,800–50,000 eggs, thus the ten initial founders may have determined the genetic composition of subsequent generations. Our findings of population bottleneck, small effective population size, and significant divergence from Lake Tuendae suggest that the low diversity of the Camp Cady population is probably a lasting consequence of the original founding event in 1986.

MC Spring is an artificial habitat that appears to have been excavated from a seep area long ago, as evidenced by vegetated spoil piles remaining alongside. The spring last connected with the Mojave River via Soda Lake during the flood of 1934, and cannot have experienced immigration of Mohave tui chub by natural means since floodwaters receded. MC Spring is less than 10 m² in size, consists of a single pool with no outlet, and has a low carrying capacity. Water temperatures remain near 15 °C and dissolved oxygen varies from around 5 mg/L near the surface to zero at 1.5 m, near the bottom. Recent counts indicate that the MC Spring population consists variably of 257–618

individuals, with the largest five individuals accounting for 10–20 % of the total biomass (authors' unpublished data). Fecundity is related to body mass, and its unequal distribution is expected to reduce effective population size. Thus, the MC Spring population's low allelic richness, low heterozygosity, and divergence from other populations likely result from genetic drift associated with independent founding events, persistent small effective population size, compounded by disproportionate reproductive contributions made by a few large-bodied individuals in the population.

Genetic management for the endangered Mohave tui chub

Our analyses suggest that genetic drift at Camp Cady and MC Spring has influenced global genetic structure of the Mohave tui chub. However, this study did not examine whether selection and local adaptation may have contributed to the development of genetic structure. Conservation biologists often invoke the preservation of evolutionary potential as an important goal toward assuring future adaptability and species viability (Franklin 1980). Mohave tui chubs are fecund, omnivorous, long-lived generalists with the plasticity to acclimatize to a broad range of environments. We suggest that the most efficient way to safeguard future viability is to prioritize preservation of the existing breadth of neutral genetic variation, using microsatellite diversity as a guide. Despite intentional redundancy in the number of refuges, the prospective lifespan of each specific population is unpredictable. We recommend the following five management actions to maintain the highest possible global genetic variation, by maximizing genetic diversity within each of the refuge populations.

- (1) Achieve the Recovery Plan goal to increase the number of viable refuge populations to a minimum of six. Two new additional populations have very recently been established at Deppe Pond (on the grounds of the Lewis Center for Educational Research) and at Morningstar Mine Pond (in Mojave National Preserve). In addition to requiring a minimum population size for recovery populations, the Recovery Plan specifies that each must be self-maintaining for a period of 5 years before reclassification to threatened status may be considered. Due to the observed high failure in translocated Mohave tui chub populations, the total number of refuges should exceed the required minimum of six. This will improve the chances of having six viable populations and subsequent down-listing.
- (2) Implement a program to interchange individuals among populations to restore genetic variation in

each of the Mohave tui chub populations. This method was recognized by the Recovery Plan (U.S. Fish and Wildlife Service 1984), but never implemented. Between one and ten migrants per generation may be sufficient to maintain allelic diversity while not disrupting the potential for local adaptation (Mills and Allendorf 1996; Storfer 1999). We suggest exchanging ten age two or older individuals annually between Lake Tuendae (the original source population) and each derived refuge population.

- (3) No change is recommended in the current management of MC Spring. However, during periods when the population of age one or older fish in MC Spring drops below 100 individuals, managers should consider (a) removing the largest individuals to better distribute reproductive output among reproducing adults, and moving the culled fish to other populations, (b) introducing one migrant per year from Lake Tuendae, and (c) replacing floating artificial spawning substrate which has previously helped encourage spawning success and recruitment. These measures may help maintain a larger effective population size, preserving genetic diversity.
- (4) Establish new populations with at least 400 founders, drawing upon both of the genetically more robust populations at Lake Tuendae and China Lake. China Lake population was established with 400 founders of mixed sizes, which resulted in a population with similar levels of diversity to the original. Individuals selected for future translocations should belong to the age 1+ year class to reduce the potential of a few fish dominating contribution to future generations, and to minimize any potential impact to the donor populations.
- (5) Genetic diversity and effective population size should be monitored to determine the effectiveness of the recommended measures in genetically replicating donor population(s) and retention of diversity over time.

Due to technical and political constraints, we believe it is presently impossible to attain the Recovery Plan goal to "Delist...upon successful reintroduction and establishment of viable Mohave tui chub populations into a majority of its historic habitat in the Mojave River." Seventy-six years of observation indicates that the presence of arroyo chub is incompatible with maintaining populations of tui chub in the lowland reaches of the Mojave River. Removal of arroyo chubs from historic Mohave tui chub habitat by fish pesticide such as rotenone would require treatment of the entire Mojave River and most of its tributaries. We suggest such a project is untenable due to the scale of the area, proximity of a large urban populace, and the number of

drinking water reservoirs and sport fisheries which would be impacted. Moreover, interconnection of the Mojave River with the 715 km long California Aqueduct guarantees post-treatment reinvasion by non-native fishes. Among these unintentional introductions, hitch (*Lavinia exilicauda*) have recently spread and appear to interbreed with arroyo chub throughout the Mojave River (authors' unpublished data).

Although restoration to the historical habitat may be precluded, alternatives may exist to establish Mohave tui chubs in upstream tributaries, ponds, and impoundments. Almost the entire water supply of the Mojave River originates in the mountainous western part of the watershed, where waterfalls and dams create potential experimental management zones by hindering the upstream spread of invasive fishes. We suggest assessing potential management zones by evaluating locations of quality habitat, the upper limits of existing fish populations (especially arroyo chub), and barriers to upstream fish passage. Once experimental management zones are identified, removing non-native fishes and replacing them with Mohave tui chubs should be considered. Such interventions to naturally fishless waters may be independently justified as assisted migration, as nonnative species preclude reintroduction, water supplies dwindle, and habitats change in response to climate change (McLachlan et al. 2007). However, in some areas we anticipate it will not be practicable to eradicate arroyo chubs. To assess the potential for coexistence of Mohave tui chub and arroyo chub, pond and laboratory experiments should be conducted to determine fertility and potential outbreeding depression in F1 and subsequent crosses. If barriers to introgression are found, in situ field trials of interspecies competition should be attempted in headwater management zones. Developing practical methods to replace arroyo chubs within these zones will increase the possibility of establishing viable Mohave tui chub populations outside of intensively managed refuges. There may ultimately be social and institutional resistance to experimental introductions; however, without the biological tools in place, recovery will never progress beyond the existing husbandry of a small number of artificial refuge ponds.

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