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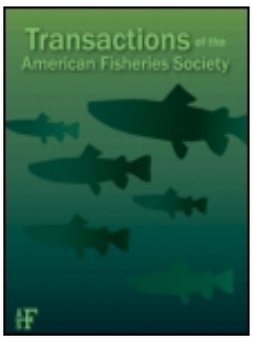

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ARTICLE

Conservation Genetics of an Urban Desert Fish, the Arroyo Chub

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Abstract

Urbanization, habitat degradation, fragmentation, and invasive species have led to the severe decline or extirpation of many endemic southern California freshwater fish species, including the Arroyo Chub *Gila orcuttii*, which has declined precipitously in recent years. Classified by the California Department of Fish and Wildlife as a species of high concern, the Arroyo Chub is native to the Los Angeles, San Gabriel, San Luis Rey, Santa Ana, and Santa Margarita rivers and Malibu and San Juan creeks. To examine Arroyo Chub population structure and genetic diversity within the species' native range, we used 10 microsatellite markers to genotype 259 individuals. We observed moderate to high genetic diversity and population differentiation both between and within drainages; Bayesian clustering supported eight distinct clusters of Arroyo Chub corresponding to eight isolated populations. Of these populations, the Big Tujunga Creek population (Los Angeles River) was the least genetically differentiated (genetic differentiation index $F_{ST} = 0.048–0.208$) and also had the highest genetic diversity (observed heterozygosity $H_o = 0.890$). Populations in Malibu Creek, Pacoima Canyon (Los Angeles River), and the Santa Margarita River were the most genetically differentiated ($F_{ST} = 0.163–0.400$), had the lowest genetic diversity ($H_o = 0.556–0.680$), and showed evidence of past bottlenecks. Arroyo Chub at these localities are at risk for continued loss of genetic diversity due to drift and small population sizes; therefore, we suggest that in the event of extirpation, translocations from the most closely related source populations should be considered. However, we recommend that management efforts focus on improving habitat quality and habitat area for Arroyo Chub in order to maximize population genetic diversity and adaptive potential over time.

With the continuing rise in human population density, urbanization poses an increasing threat to the well-being of many ecologically important endemic taxa. Multiple empirical studies have documented the anthropogenic factors leading to habitat loss and fragmentation, which in turn cause reductions in biodiversity (Fahrig 2003; Vörösmarty et al. 2010). However, maintaining biodiversity is essential to protecting both the functionality and the productivity of ecosystems (Hedrick and Miller 1992). With regard to species in highly urbanized environments, genetic surveys provide useful insights for management and conservation efforts. For instance, urbanization has been shown to reduce genetic

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variation and impact gene flow in a number of aquatic vertebrate species (Bessert and Orti 2008; Kobayashi et al. 2013; Munshi-South et al. 2013; Emel and Storfer 2015). By elucidating population structure and identifying populations with reduced genetic diversity, managers can determine the localities where populations (1) are in greatest need of conservation efforts or (2) have experienced bottlenecks or inbreeding (Frankham et al. 2010). Populations that have undergone bottlenecks and that have low effective population sizes (N_e) are more likely to experience genetic drift, causing further reduction in genetic diversity and possibly reducing evolutionary potential (Moritz 1999). As a result, such populations are candidates for increased management efforts, including habitat restoration, the designation of evolutionarily significant units or management units, the removal of nonnative species and hybrids, and reintroductions or translocations; genetic monitoring is often used to evaluate the effects of these actions on genetic diversity (Moritz 1994, 1999; Schwartz et al. 2006; Van Doornik et al. 2011; Osborne et al. 2012).

One fish species that is in need of genetic analysis is the Arroyo Chub *Gila orcuttii* (Eigenmann and Eigenmann 1890), a once-common cyprinid that over the past decade has declined in many portions of its native range, which encompasses coastal streams of southern California (J. O'Brien, personal observation). Due to stressors related to urbanization and interactions with nonnative species, the California Department of Fish and Wildlife (CDFW) classifies the Arroyo Chub as a "species of high concern"; this status rating is assigned to taxa with a high risk of becoming a critical concern due to significantly reduced range, significantly reduced abundance, and projected vulnerability over the short term (<10 generations; Moyle et al. 2015). Despite the Arroyo Chub's decline, it only qualifies for listing as a "species of moderate concern" when its entire range is considered, as Arroyo Chub also thrive in the Santa Ynez, Santa Maria, Cuyama, Santa Clara, and Mojave River systems and other small coastal streams—waters that are outside the species' native range (Moyle et al. 2015). However, because introduced Arroyo Chub are known to hybridize with other cyprinids, introduced populations may be introgressed; without thorough genetic analysis, fish from such populations would be unacceptable for use in translocations or reintroduction to the native range (Hubbs and Miller 1943; Greenfield and Deckert 1973; Swift et al. 1993; Moyle et al. 1995).

Preservation of the Arroyo Chub requires an understanding of threats that are present in the native range, which includes the Los Angeles, San Gabriel, San Luis Rey, Santa Ana, and Santa Margarita rivers and Malibu and San Juan creeks (Wells and Diana 1975). Because the species' native range overlaps with the greater Los Angeles area—a region with one of the greatest human population densities in North America—the Arroyo Chub faces habitat degradation and fragmentation resulting from the dramatic increase in urbanization over the past century. In these areas, human development has nearly eliminated the lower-gradient streams that provide ideal habitat for Arroyo Chub, as most of these streams have been channelized, dammed, diverted, and otherwise degraded (Moyle et al. 2015). The present-day population structure of Arroyo Chub is likely affected by (1) these watercourse alterations, which reduce connectivity between native watersheds by preventing the floods that historically provided such connection; and (2) dams, which fragment populations within a given watershed. Dams are known to alter flows, impair sediment recruitment, and create barriers that prevent genetic exchange between chub populations; dams have been linked to reduced diversity in other fish species as well (Bessert and Orti 2008; Liermann et al. 2012; Moyle et al. 2015). Human modification of watercourses via logging, mining, flood control, and water storage projects has drastically changed the character of streams, and the recent drought in California has continued to reduce the amount of available habitat (Swift et al. 1993).

The combined negative effects of invasive species and habitat loss, degradation, and fragmentation have substantially reduced Arroyo Chub populations, highlighting the need for genetic analysis. We used microsatellite data from Arroyo Chub populations across the species' native range to analyze population structure and genetic diversity, examine potential barriers to dispersal, and determine the number of distinct populations that could serve as management units. We recommend appropriate conservation management strategies and discuss source populations that could be used for translocations.

METHODS

Sample collection.—Samples were collected from 25–66 Arroyo Chub within each of six native drainages (sampling efforts were unsuccessful in the seventh drainage, the San Luis Rey River): Malibu Creek (MC), Los Angeles River (LA), San Gabriel River (SG), Santa Ana River (SA), San Juan Creek (SJ), and Santa Margarita River (SM; Table 1; Figure 1). Arroyo Chub were captured by use of backpack electrofishing, seining, and dipnetting. The fish were collected whole, or the upper caudal fin was clipped and stored in a 2-mL microcentrifuge tube containing a 95% solution of ethanol. Whole genomic DNA was extracted from each fish by using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, California) in accordance with the manufacturer's protocols.

Microsatellite genotyping.—Using previously published procedures, we genotyped each Arroyo Chub at 10 microsatellite loci: *Pmac01, Pmac04, Pmac15, Pmac21, Pmac24, Pmac29*, and *Pmac32* (Mahardja et al. 2012); *Cyp-G3* and *Cyp-G48* (Baerwald and May 2004); and *Gbi-G13* (Meredith and May 2002). For genotyping, 2 μL of PCR product were added to 0.28 μL of Applied Biosystems, Inc. (ABI), GeneScan 500 LIZ size standard and 8.72 μL of Hi-Di formamide (Life Technologies [LT], Carlsbad, California) in individual wells on a 96-well plate. Samples were denatured at 95°C for 3 min and then were electrophoresed on an ABI 3730XL

TABLE 1. List of California watersheds, sample collection sites, GPS coordinates, collection year, and number of Arroyo Chub (*N*) that were sampled at each site.

Watershed	Collection site	GPS coordinates	Year	*N*
Malibu Creek (MC)	Las Virgenes Creek	34.09680, −118.72845	2013	19
	Above Serra Road Bridge	34.04722, −118.68972	2012	5
	Above Rindge Dam	34.07640, −118.70230	2012	11
	Near Cross Creek Road Bridge	34.04539, −118.68703	2013	7
Los Angeles River (LA)	Pacoima Canyon (PC)	34.34541, −118.35827	2013	20
	Big Tujunga Creek (BTC)	34.29451, −118.24232	2013	20
		34.30181, −118.25575	2012	20
San Gabriel River (SG)	West Fork (WF)	34.24319, −117.87497	2013	24
		34.24317, −117.92865	2013	2
	Walnut Creek (WC)	34.08722, −117.84511	2013	40
Santa Ana River (SA)	Santa Ana River	34.03594, −117.35670	2013	40
San Juan Creek (SJ)	Bell Canyon–Starr Ranch	33.63169, −117.55531	2013	24
	Hot Springs Creek	33.60814, −117.51082	2013	1
Santa Margarita River (SM)	Temecula Creek	33.43408, −116.85529	2013	26
Total *N*				259

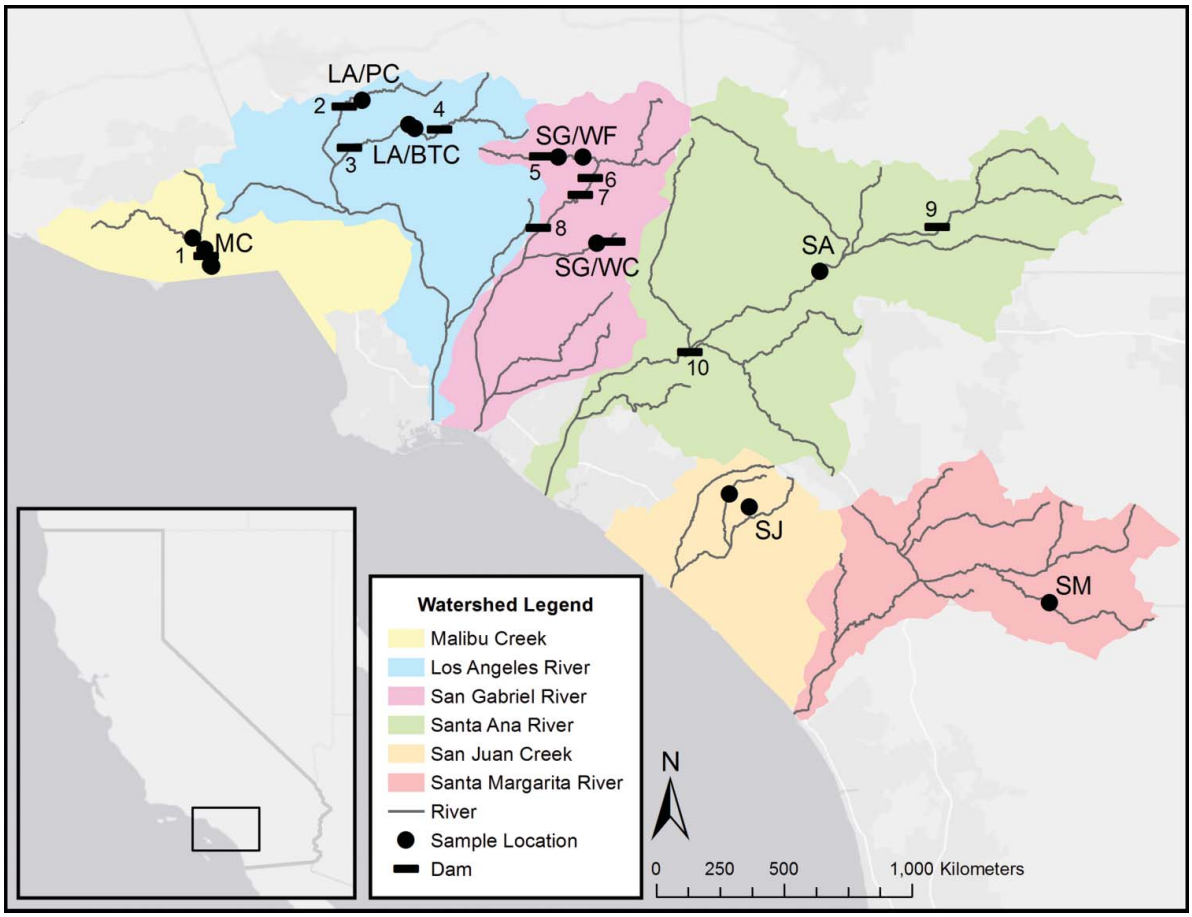

FIGURE 1. Map of southern California, depicting the major rivers in the Arroyo Chub's native range, watershed boundaries (eight-digit hydrologic unit data set; U.S. Geological Survey), sampling locations, and major dams (1 = Rindge Dam; 2 = Pacoima Dam; 3 = Hansen Dam; 4 = Big Tujunga Dam; 5 = Cogswell Dam; 6 = San Gabriel Dam; 7 = Morris Dam; 8 = Santa Fe Dam; 9 = Seven Oaks Dam; 10 = Prado Dam). Location codes are defined in Table 1.

DNA Analyzer (LT). The resulting peaks were analyzed using GENEMAPPER version 4.0 (LT). Electropherograms were inspected twice to confirm allele sizes; individuals that were run on multiple plates had consistent scores across the different runs. Samples with poor genotypic quality (<70% of the genotypic data) were discarded from further analysis. We used MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) to detect abnormal values in genotypic data, as such values potentially resulted from stuttering or the presence of null alleles (indicated by significant homozygote excess).

Population structure.—We used STRUCTURE version 2.3.3 (Pritchard et al. 2000) to determine the optimal number of clusters (K) and to assign individual Arroyo Chub to groups. For each K-value from 1 to 10, we ran three iterations with a 100,000-replicate burn-in period and 1,000,000 Markov chain–Monte Carlo replications. To determine the optimal value of K, STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to calculate ΔK (Evanno et al. 2005). The software CLUMPP (Jakobsson and Rosenberg 2007) and the Greedy K algorithm were employed to test for multimodality; the three STRUCTURE outputs for optimal K were compiled for graphical representation via the program DISTRUCT (Rosenberg 2004). The program GENETIX (Belkhir et al. 2003) was used to develop a graphical representation of genetic divergence through factorial correspondence analysis.

Genetic diversity.—Samples from each locality were analyzed to assess genetic diversity, estimate N_e, and detect bottlenecks. We tested for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) by using GENEPOP version 4.2 (Raymond and Rousset 1995). A sequential Bonferroni correction ($\alpha = 0.05$) was applied to detect the significance of HWE and LD results. The number of private alleles (N_P), allelic frequencies, observed heterozygosity (H_o), and expected heterozygosity (H_e) were calculated by using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). We used HP-RARE (Kalinowski 2005) to calculate allelic richness (A_r) and private allelic richness (A_P); these genetic diversity measures use rarefaction to correct for the increased likelihood of detecting rare alleles in larger sample sizes (Kalinowski 2004). Values of A_r and A_P were calculated based on the minimum number of genomic copies (i.e., $N = 32$) found for any locus. Pairwise values of the genetic differentiation index F_{ST} were calculated using FSTAT version 2.9.3.2 (Goudet 1995), and P-values were obtained after 560 permutations. A Bonferroni correction to the α value (0.05) was used to determine the significance of F_{ST} values.

Population bottlenecks and effective population size.—We used two tests to detect population bottlenecks: (1) Wilcoxon's signed rank test for excess heterozygosity (H_k; Cornuet and Luikart 1996) was conducted with BOTTLENECK version 1.2.02 (Piry et al. 1999); and (2) the M-ratio test (Garza and Williamson 2001) was implemented in the program M_P_Val (National Oceanic and Atmospheric Administration–Fisheries; swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298). First, to detect the probability of a more recent population bottleneck, the H_k test was performed by using Wilcoxon's two-tailed test for heterozygote excess or heterozygote deficiency with 5,000 replications. Two microsatellite mutation models were applied: the stepwise mutation model (SMM) and the two-phase model (TPM; Di Rienzo et al. 1994). The TPM parameters were 12% variance, 95% stepwise mutations, and 5% non-stepwise mutations, as recommended by Piry et al. (1999). Second, we calculated the M-ratio as the mean ratio of the number of alleles (k) over the range (r) of allele sizes (base pairs). A smaller-than-expected M-ratio indicates that a population likely has experienced a severe genetic bottleneck (Garza and Williamson 2001). Calculation of M was based on the following parameters (recommended by Garza and Williamson 2001): the proportion of one-step mutations (p_s) was 0.9; the average size of non-one-step mutations (delta_g) was 3.5; and $\theta = 4N_{e\mu}$ (where N_e = effective population size and μ = mutation rate) was 10.

We calculated N_e by using the program N_eEstimator version 2.01 (Do et al. 2014) and implementing the LD method (Waples and Do 2008), which assumes random mating. We used a $P_{critical}$ of 0.02 for populations with sample sizes greater than 25 and a $P_{critical}$ of 0.03 when sample size was 25 or lower.

RESULTS

MICRO-CHECKER detected possible null alleles at *Pmac24* in the LA/Pacoima Canyon and SJ samples; *Pmac01* in the SG/Walnut Creek samples; *Pmac29* in the SG/West Fork samples; *Pmac04* in the SA samples; and *Pmac32* in the SM samples. The MC, LA/Big Tujunga Creek, and SA populations exhibited significant deviations from HWE expectations ($P \leq 0.05$). In the MC population, only *Pmac01* significantly deviated from HWE ($P < 0.05$), and the deviation remained significant after Bonferroni correction ($\alpha = 0.05$). For the LA/Big Tujunga Creek population, *Pmac01* and *Pmac24* were the only loci that showed significant deviations from HWE ($P < 0.05$); after sequential Bonferroni adjustment ($\alpha = 0.05$), only *Pmac01* remained significant. In the SA samples, three loci (*Pmac04, Pmac21*, and *Pmac24*) were identified as deviating from HWE ($P < 0.05$); however, only *Pmac04* showed significant deviation after Bonferroni correction ($\alpha = 0.05$). Out of 360 tests for LD, 19 locus pairs were detected as demonstrating significant LD ($P < 0.05$); after the Bonferroni correction was applied, only six locus pairs remained significant (*Pmac15–Pmac32* for LA/Pacoima Canyon; *Pmac15–Pmac04* and *Pmac29–Pmac24* for LA/Big Tujunga Creek; *Cyp-G3–Gbi-G13* for SG/West Fork; and *Pmac01–Gbi-G13* and *Pmac32–Gbi-G13* for SJ). Because deviations from HWE or LD showed no consistent pattern across populations or loci, all loci were retained for further analysis.

Population Structure

Based on STRUCTURE analysis, the optimal K-value was 8, reflecting the following independent clusters: (1) MC, (2)

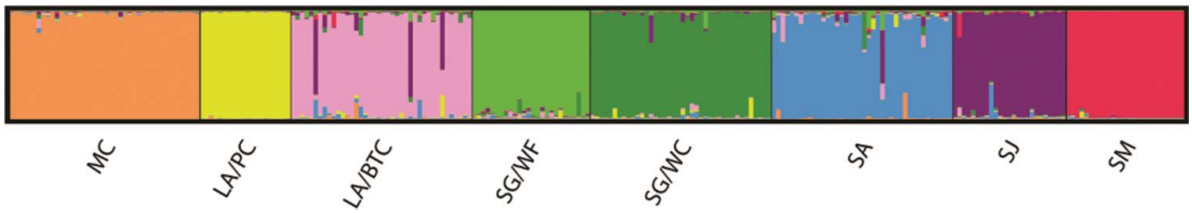

FIGURE 2. DISTRUCT bar plot with eight clusters ($K = 8$), showing Arroyo Chub population substructure among sampling sites (MC = Malibu Creek; LA/PC = Los Angeles River/Pacoima Canyon; LA/BTC = Los Angeles River/Big Tujunga Creek; SG/WF = San Gabriel River/West Fork; SG/WC = San Gabriel River/Walnut Creek; SA = Santa Ana River; SJ = San Juan Creek; SM = Santa Margarita River). Each vertical bar represents a single individual while the height of a color indicates probability of assignment to that cluster.

LA/Pacoima Canyon, (3) LA/Big Tujunga Creek, (4) SG/West Fork, (5) SG/Walnut Creek, (6) SA, (7) SJ, and (8) SM (Figure 2). The factorial correspondence analysis revealed a central cluster of more genetically similar populations (Figure 3). Arroyo Chub from the more central localities (LA/Big Tujunga Creek, SG/West Fork, SG/Walnut Creek, SA, and SJ) clustered more closely together, whereas fish from MC, LA/Pacoima Canyon, and SM exhibited greater separation from this central cluster.

Pairwise F_{ST} values ranged from 0.048 to 0.400 (Table 2), and all values were significant after correction for multiple tests ($P < 0.002$). Samples from LA/Pacoima Canyon and SM showed the greatest genetic differentiation, but the F_{ST} values for LA/Pacoima Canyon–MC and for MC–SM indicated that those pairs of populations were also quite distinct ($F_{ST} > 0.300$; Table 2). Populations in SA and LA/Big Tujunga Creek were the least differentiated ($F_{ST} = 0.048$; Table 2). Pairwise F_{ST} values between the central populations (LA/Big Tujunga Creek, SG/West Fork, SG/Walnut Creek, SA, and SJ) were lower than values for the edge groups but still indicated significant differentiation ($F_{ST} < 0.110$; Table 2).

Genetic Diversity

The average number of alleles per locus (N_A) ranged from 3.80 to 15.10, and the average N_A across all populations was 9.86 (Table 3). *Pmac01* was monomorphic in the LA/Pacoima Canyon samples. Values of H_e ranged from 0.543 to 0.890, and H_o ranged from 0.556 to 0.890 (Table 3). The LA/Pacoima Canyon and SM populations had the lowest heterozygosity, whereas the LA/Big Tujunga Creek population exhibited the highest levels of heterozygosity (H_o and $H_e = 0.890$). The average N_P was 6.25 (Table 3). The MC and SA populations showed the highest N_P (12 in each case). In contrast,

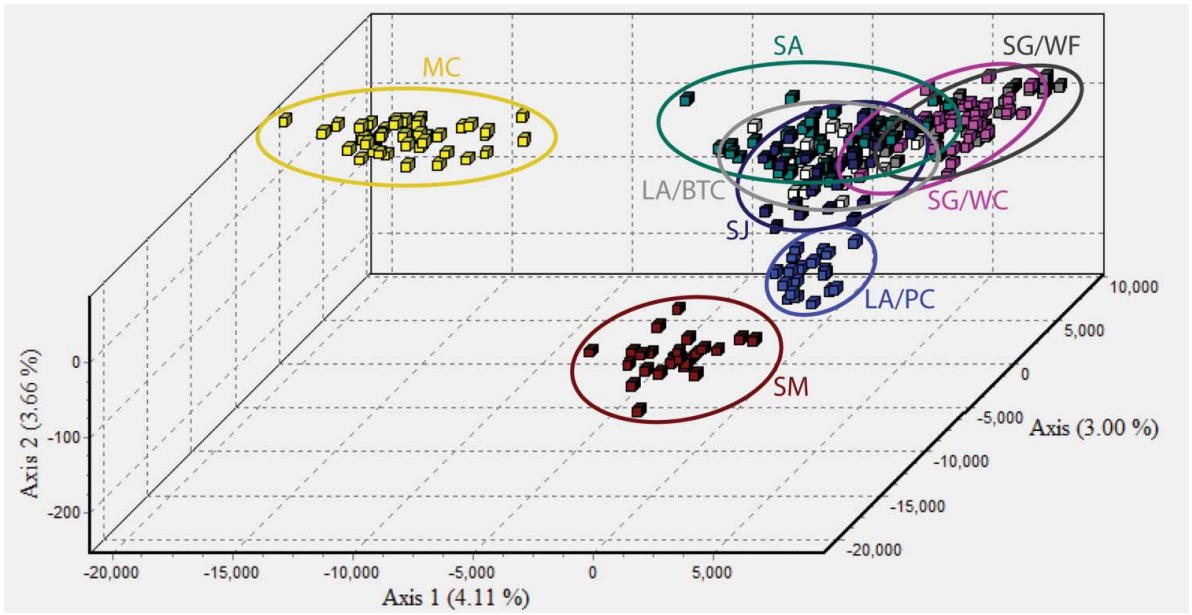

FIGURE 3. Orthogonal plot illustrating factorial correspondence analysis of individual Arroyo Chub (as implemented in GENETIX), with fish grouped in the populations identified by STRUCTURE analysis. The three principal axes explain the degree of genetic variation between individuals. Each square represents an individual from a particular site: Malibu Creek (MC; yellow), Los Angeles River/Pacoima Canyon (LA/PC; blue), Los Angeles River/Big Tujunga Creek (LA/BTC; white), San Gabriel River/West Fork (SG/WF; gray), San Gabriel River/Walnut Creek (SG/WC; fuchsia), Santa Ana River (SA; teal), San Juan Creek (SJ; navy), and Santa Margarita River (SM; maroon).

TABLE 2. Pairwise values of the genetic differentiation index F_{ST} calculated for Arroyo Chub populations. All F_{ST} values presented here are significant ($P < 0.002$ with Bonferroni correction for multiple tests). Collection site codes are defined in Table 1.

Location	MC	LA/PC	LA/BTC	SG/WF	SG/WC	SA	SJ
MC							
LA/PC	0.354						
LA/BTC	0.167	0.188					
SG/WF	0.217	0.246	0.068				
SG/WC	0.217	0.235	0.064	0.070			
SA	0.163	0.223	0.048	0.071	0.086		
SJ	0.189	0.246	0.058	0.101	0.073	0.073	
SM	0.302	0.400	0.208	0.238	0.238	0.215	0.199

only one private allele was detected in the LA/Pacoima Canyon population, and no private alleles were found in the SM samples. See Supplementary Table S.1 (available in the online version of this paper) for allele frequencies at each locus.

Population Bottlenecks and Effective Population Size

Under both the TPM and SMM models, the H_k test indicated that the MC population underwent a bottleneck (TPM: $P = 0.002$; SMM: $P = 0.001$; Table 4). Additionally, the SG/Walnut Creek and SA populations showed evidence of bottlenecks under the SMM model ($P = 0.014$) but not under the TPM model. The *M*-ratio test provided evidence for bottlenecks in the following populations: LA/Pacoima Canyon ($P < 0.001$), SG/West Fork ($P < 0.001$), SG/Walnut Creek ($P = 0.002$), SJ ($P = 0.024$), and SM ($P < 0.001$; Table 4). Estimated N_e ranged from 5.8 to infinity (Table 4). The 95% confidence intervals for N_e tended to be wide, and four of the eight populations had infinity as an upper confidence limit, indicating that in combination with our low sample sizes, we had low power for estimating N_e by use of the LDN_e method (Waples and Do 2010).

DISCUSSION

We observed a high level of Arroyo Chub population differentiation both within and between the native drainages; this is likely a result of barriers to gene flow (e.g., dams) as well as historical and contemporary watershed boundaries. Overall, the Arroyo Chub populations were each observed to be genetically distinct, and they exhibited genetic diversity that was average or high in comparison with the diversity that has been reported for other freshwater fishes.

Population Structure

Our STRUCTURE results suggested that the MC, LA/Pacoima Canyon, LA/Big Tujunga Creek, SG/West Fork, SG/Walnut Creek, SA, SJ, and SM populations are all distinct. Population fragmentation in combination with genetic drift was likely responsible for generating the observed population structure, as the two LA populations (Pacoima Canyon and Big Tujunga Creek) and the two SG populations (West Fork and Walnut Creek) were genetically distinct despite occupying the same watershed. The pattern of population fragmentation likely stems from dams and other migration barriers, such as culverts,

TABLE 3. Expected heterozygosity (H_e), observed heterozygosity (H_o), average number of alleles (N_A) across all loci, allelic richness (A_r), number of private alleles (N_P), private allelic richness (A_P), and effective population size (N_{eLD} [calculated via the linkage disequilibrium method]; with 95% confidence interval [CI]) for Arroyo Chub after data were jackknifed over loci. Collection site codes are defined in Table 1.

Location	N	H_e	H_o	N_A	A_r	N_P	A_P	N_{eLD} (95% CI)
MC	42	0.676	0.680	9.00	7.21	12	1.22	171 (55.7–∞)
LA/PC	20	0.543	0.604	3.80	3.71	1	0.16	5.8 (2.9–10.2)[a]
LA/BTC	40	0.890	0.890	15.10	11.82	7	0.79	149 (94.7–325.5)
SG/WF	26	0.825	0.819	10.30	9.05	2	0.48	38.1 (27.7–57.5)
SG/WC	40	0.820	0.815	10.70	8.63	8	0.68	2,122.3 (198–∞)
SA	40	0.871	0.847	14.60	11.36	12	0.83	∞ (198.3–∞)
SJ	25	0.845	0.854	11.30	10.23	8	0.99	39.7 (26–73.4)[a]
SM	26	0.580	0.556	4.10	3.84	0	0.06	∞ (42.2–∞)
Mean		0.756	0.758	9.86	8.23	6.25	0.65	

[a]The N_{eLD} was calculated using a $P_{critical}$ of 0.03 when N was 25 or lower. For all other populations ($N > 25$), $P_{critical}$ was 0.02.

TABLE 4. Results of the *M*-ratio test and the heterozygosity excess (H_k) test used to detect bottlenecks in Arroyo Chub populations (TPM = two-phase model; SMM = stepwise mutation model). Significant values are shown in bold (NS = not significant). Collection site codes are defined in Table 1.

Location	*M*	*P*-value	H_k model significance
MC	0.790	0.558	**TPM ($P = 0.002$); SMM ($P = 0.001$)**
LA/PC	0.498	<0.001	NS
LA/BTC	0.752	0.274	NS
SG/WF	0.521	<0.001	NS
SG/WC	0.618	0.002	**SMM ($P = 0.014$)**
SA	0.738	0.218	**SMM ($P = 0.014$)**
SJ	0.642	0.024	NS
SM	0.548	<0.001	NS

drop structures, and dry reaches, which either eliminate or drastically reduce the potential for genetic exchange between Arroyo Chub populations. Specifically, within the LA drainage, the Pacoima Dam in Pacoima Canyon and the Hansen Dam in Big Tujunga Creek serve as historical and contemporary barriers isolating the two LA Arroyo Chub populations from each other as well as from the remainder of the drainage. Similarly, in SG, barriers such as the Santa Fe, Morris, and San Gabriel dams separate the West Fork and Walnut Creek populations and have likely prevented interbreeding and gene flow since the 1920s. Alternatively, introductions of Arroyo Chub from other watersheds may have contributed to the observed differentiation within the LA and SG systems, but analyses based on other genetic markers or comparisons with historical samples would be necessary to elucidate such relationships.

We only observed population fragmentation within the LA and SG drainages. This difference may be due to the limited number of individuals sampled from SA and SM, as genetic structure analysis was based on fish collected from only one site in each of those rivers. Because migration barriers similarly fragment SA and SM, genetic analysis of individuals from more distant sections of those drainages could reveal fragmentation levels that are comparable to those observed in the LA and SG populations. Unfortunately, drought conditions as well as the general decline of native Arroyo Chub populations prevented us from finding fish at a wide variety of sampling sites. In fact, no Arroyo Chub were found in the seventh native drainage—the San Luis Rey River. Thus, extirpation of the San Luis Rey River population may have already occurred.

Historical and contemporary watershed boundaries can largely explain the genetic differentiation between Arroyo Chub populations. Of the eight populations we analyzed, the MC, LA/Pacoima Canyon, and SM populations had the highest pairwise F_{ST} values, suggesting that they were the most genetically distinct from each other as well as distinct from the other populations. These three populations represent the edge groups: MC and LA/Pacoima Canyon are the northernmost locations in the native range, and SM is the southernmost location. Vandergast et al. (2007) provided a map suggesting that during the Quaternary Period, the lower reaches of LA, SG, and SA were once inundated, thus connecting the populations. Although portions of LA, SG, and SA are now highly fragmented, these rivers share a common mouth, and large flood events taking place as recently as the 20th century caused the rivers to spill their banks and intermix, likely facilitating the migration of Arroyo Chub among these more centrally located drainages (J. O'Brien, personal observation). Analysis with different genetic markers would be necessary to confirm these historical relationships; however, the past watershed boundaries in combination with the observed genetic differentiation indicate that Arroyo Chub population structure among MC, SM, and the central watersheds is more ancient, whereas substructure among populations in the central watersheds has emerged more recently.

Genetic Diversity

Arroyo Chub populations exhibited a moderate to high level of genetic diversity. The mean H_e for Arroyo Chub (0.756) exceeded the mean heterozygosity reported for most freshwater fishes (0.54; DeWoody and Avise 2000). The Arroyo Chub populations with the lowest pairwise F_{ST} values (LA/Big Tujunga Creek, SA, SG/West Fork, SG/Walnut Creek, and SJ) also demonstrated the highest genetic diversity. The low pairwise F_{ST} values suggested that these five centrally located populations have experienced greater and/or more recent gene flow than populations at the distributional edge, thus enabling better preservation of genetic diversity and reducing genetic differentiation (Allendorf 1983; Epps et al. 2005). Because larger populations maintain genetic diversity better than small populations, river size and population size may also explain the observed differences in genetic diversity between Arroyo Chub populations. The largest river in the Arroyo Chub's native range is SA, followed by SG and LA. The larger sizes of these rivers may partially explain the higher genetic diversity observed among these Arroyo Chub populations, although it is difficult to quantify the proportion of suitable habitat and the degree of fragmentation occurring in each drainage. Furthermore, while there is very little comprehensive survey data on Arroyo Chub, the LA/Big Tujunga Creek and SA populations are known to have been relatively large

historically, and LA/Big Tujunga Creek appears to contain the largest and most robust population at present (J. O'Brien, personal observation). Although the SJ population is smaller now, it was also large historically (J. O'Brien, personal observation). Thus, the most genetically diverse populations of Arroyo Chub are also the largest, allowing them to maintain more diversity over time (Frankham 1996). These five populations (LA/Big Tujunga Creek, SA, SG/West Fork, SG/Walnut Creek, and SJ) all had H_e and H_o values above 0.80, which is greater than the heterozygosity observed in a sympatric species, the Santa Ana Speckled Dace *Rhinichthys osculus* subsp. ($H_e = 0.65$; Nerkowski 2015). Like the Arroyo Chub, the Santa Ana Speckled Dace has drastically declined in abundance due to anthropogenic destruction of habitat and the effects of invasive species. The CDFW classifies the Santa Ana Speckled Dace as a species of critical concern; populations still remain in the SA and SG watersheds, whereas the LA population was extirpated (Moyle et al. 1995, 2015).

The LA/Pacoima Canyon and SM populations of Arroyo Chub exhibited the lowest genetic diversity observed among the sampled populations. The significant *M*-ratio tests indicated that historical bottlenecks were more severe in the LA/Pacoima Canyon and SM populations, which may account for their reduced genetic diversity and low N_e values. A population with a low N_e is subject to increased levels of genetic drift, which reduces genetic diversity; populations with low genetic diversity may experience a loss of evolutionary potential (Reed and Frankham 2003). In particular, the LA/Pacoima Canyon population had the lowest N_e (5.8 fish) observed among the study populations, suggesting that it is highly susceptible to a continued loss of genetic diversity through genetic drift. Although the LD method lacked the necessary power to determine the N_e for SM, the lower 95% confidence limit (42.4 fish) was still far below the N_e thresholds (500 individuals: Franklin 1980; 5,000 individuals: Lande 1995) recommended for population maintenance.

The reduced genetic diversity, population bottlenecks, and low N_e values in Arroyo Chub populations are most likely attributable to habitat loss. Arroyo Chub once occupied a 32.2–48.3-km (20–30-mi) range in LA/Pacoima Canyon; however, CDFW biologists only found the fish in a 0.402-km (0.25-mi) section of wetted habitat within the canyon. Similarly, during sampling conducted in SM from the ocean to Temecula Creek, CDFW biologists only found Arroyo Chub in a small pool within Temecula Creek. In both LA/Pacoima Canyon and SM, Arroyo Chub were congregated together in one section of wetted habitat, suggesting that habitat degradation, habitat fragmentation, and drought are responsible for the reduced number and decreased genetic diversity of fish in these populations.

Conservation Implications

Due to the high level of genetic distinctiveness of Arroyo Chub from the different drainages, we recommend that conservation efforts recognize the following eight populations as separate management units: MC, LA/Pacoima Canyon, LA/Big Tujunga Creek, SG/West Fork, SG/Walnut Creek, SA, SJ, and SM. Due to their isolation and low census sizes, all of the remaining native populations of Arroyo Chub are vulnerable to extirpation through the combined effects of genetic diversity loss and stochastic events.

Despite the threats associated with habitat loss and invasive species, most of the Arroyo Chub populations were found to possess reasonably high levels of genetic diversity. In terms of prioritizing populations for conservation efforts, the LA/Big Tujunga Creek and SA populations are important reservoirs of genetic diversity due to their high heterozygosity, high A_r, and larger habitat size. The SG/West Fork, SG/Walnut Creek, and SJ populations also exhibited relatively high levels of genetic diversity, and they should be preserved to maintain the species' adaptive potential. The upper SG is one of the last mostly protected basins that contain Arroyo Chub, potentially making it the best location for management efforts, such as habitat preservation or restoration (O'Brien 2011).

In contrast, the LA/Pacoima Canyon and SM populations of Arroyo Chub are at the greatest risk of continued genetic diversity losses. Because the Arroyo Chub in LA/Pacoima Canyon have the lowest N_e and are isolated from the remainder of the LA drainage, they face a greater risk of inbreeding depression and the random accumulation of deleterious alleles (e.g., Gilpin and Soulé 1986; Caughley 1994; Frankham et al. 2010). In these cases, it is necessary to weigh the risks of continued genetic isolation against the potential costs of admixture among management units (Moritz 1999). Translocation of individuals could beneficially increase gene flow and might increase fitness, but it could alternatively result in outbreeding depression and a loss of adaptive diversity. However, the probability of outbreeding depression is lower for situations in which populations are located in similar environments and have experienced genetic exchange within the past 500 years (Frankham et al. 2011). Arroyo Chub are physiologically adapted to a wide range of habitats and temperature fluctuations, have proliferated when introduced outside their native range, and have experienced genetic differentiation exacerbated by drift and bottlenecks; these characteristics suggest that the need for genetic rescue currently exceeds the risk of outbreeding depression (Moyle et al. 2015). Consequently, translocations should be considered as a method of supplementing genetic diversity if the LA/Pacoima Canyon and SM populations continue to decline. If managers decide to supplement these at-risk populations, we recommend the translocation of Arroyo Chub from areas that have the highest within-population genetic diversity and the greatest genetic similarity to the receiving population. Due to genetic similarity, the LA/Big Tujunga Creek population would be the best source for translocations to LA/Pacoima Canyon. The SM population, while highly genetically distinct, is least differentiated from the SJ population. The SJ population also exhibits high genetic

diversity; therefore, it is the optimal source population for translocations to SM. However, translocations are unlikely to be successful unless conservation managers address the greatest threats facing the Arroyo Chub—habitat degradation and loss, fragmentation of populations, and the presence of invasive species. Although some streams may be permanently eradicated due to urbanization, efforts to either restore habitat or maintain the existing habitat will be essential in securing genetic stability for the Arroyo Chub.

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REFERENCES

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Genetics and Conservation 18:51–65.

Baerwald, M. R., and B. May. 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.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2003. GENETIX version 4.04, logiciel sous Windows pour la genetique des populations. [GENETIX version 4.04, Windows TM software for population genetics.] Universite de Montpellier II, Montpellier, France.

Bessert, M. L., and G. Orti. 2008. Genetic effects of habitat fragmentation on Blue Sucker populations in the upper Missouri River (*Cycleptus elongatus* Lesueur, 1918). Conservation Genetics 9:821–832.

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology 63:215–244.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

DeWoody, J. A., and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology 56:461–473.

Di Rienzo, A., A. Peterson, J. Garza, A. Valdes, M. Slatkin, and N. Freimer. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the USA 91:3166–3170.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovendon. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective size (N_e) from genetic data. Molecular Ecology Resources 14:209–214.

Earl, D. A., and B. M. Von Holdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Eigenmann, C. H., and R. S. Eigenmann. 1890. Additions to the fauna of San Diego. Processions of the California Academy of Sciences 3(1890–1892):1–24.

Emel, S. L., and A. Storfer. 2015. Landscape genetic and genetic structure of the southern torrent salamander, *Rhyacotriton variegatus*. Conservation Genetics 16:209–221.

Epps, C. W., P. J. Palsboll, J. D. Wehausen, G. K. Roderick, R. R. Ramey II, and D. R. McCullough. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecology Letters 8:1029–1038.

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

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Frankham, R. D. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2010. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the probability of outbreeding depression. Conservation Biology 25:465–475.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–149 *in* M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary–ecological perspective. Sinauer, Sunderland, Massachusetts.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum viable populations: processes of species extinction. Pages 19–34 *in* M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity 86:485–486.

Greenfield, D. W., and G. D. Deckert. 1973. Introgressive hybridization between *Gila orcutti* and *Hesperoleucus symmetricus* (Pisces: Cyprinidae) in the Cuyama River basin, California II: ecological aspects. Copeia 1973:417–427.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications 2:30–46.

Hubbs, C. L., and R. R. Miller. 1943. Mass hybridization between two genera of cyprinid fishes in the Mojave Desert, California. Papers of the Michigan Academy of Science Arts and Letters 28:342–378.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

Kalinowski, S. T. 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conservation Genetics 5:539–543.

Kalinowski, S. T. 2005. HP-Rare: a computer program for performing rarefaction on measures of allelic diversity. Molecular Ecology Notes 5:187–189.

Kobayashi, S., S. Abe, and R. Matsuki. 2013. Genetic structure of a Japanese brown frog (*Rana japonica*) population implies severe restriction of gene flow caused by recent urbanization in a satoyama landscape. Mitochondrial DNA 24:697–704.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9:782–291.

Liermann, C. R., C. Nilsson, J. Robertson, and R. Y. Ng. 2012. Implications of dam obstruction for global freshwater fish diversity. Bioscience 62:539–548.

Mahardja, B., B. May, and M. Baerwald. 2012. Characterization of 36 additional microsatellite loci in Splittail (*Pogonichthys macrolepidotus*) and cross-amplification in five other native Californian cyprinid species. Conservation Genetics Resources 4:917–921.

Meredith, E., and B. May. 2002. Microsatellite loci in the Lahontan Tui Chub, *Gila bicolor obesa*, and their utilization in other chub species. Molecular Ecology Notes 2:156–158.

Moritz, C. 1994. Defining "evolutionarily significant units" for conservation. Trends in Ecology and Evolution 9:373–375.

Moritz, C. 1999. Conservation units and translocations: strategies for conserving evolutionary processes. Hereditas 130:217–228.

Moyle, P. B., R. M. Quiñones, J. V. Katz, and J. Weaver. 2015. Fish species of special concern in California. California Department of Fish and Wildlife, Sacramento.

Moyle, P. B., R. M. Yoshiyama, J. E. Williams, and E. D. Wikramanayake. 1995. Fish species of special concern in California. Final Report to California Department of Fish and Game, Contract N-2128IF, Sacramento.

Munshi-South, J., Y. Zak, and E. Pehek. 2013. Conservation genetics of extremely isolated urban populations of the northern dusky salamander (*Desmognathus fuscus*) in New York City. PeerJ [online serial] 1:e64.

Nerkowski, S. A. 2015. Microsatellite analysis of population structure in the Santa Ana Speckled Dace (*Rhinichthys osculus*). Master's thesis. California State University, San Bernardino.

O'Brien, J. W., H. K. Hansen, and M. E. Stephens. 2011. Status of fishes in the upper San Gabriel River basin, Los Angeles County, California. California Fish and Game 97:149–163.

Osborne, M. J., E. W. Carson, and T. F. Turner. 2012. Genetic monitoring and complex population dynamic: insights from a 12-year study of the Rio Grande Silvery Minnow. Evolutionary Applications 5:553–574.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6 (Genetic analysis in Excel): population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5 (Genetic analysis in Excel): population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Heredity 90:502–503.

Pritchard, J. K., M. Stephens, and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

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

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17:230–237.

Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Schwartz, M. K., G. Luikart, and R. Waples. 2006. Genetic monitoring as a promising tool for conservation management. Trends in Ecology and Evolution 22:25–33.

Swift, C. S., T. R. Haglund, M. Ruiz, and R. N. Fisher. 1993. The status and distribution of the freshwater fishes of southern California. Bulletin of the Southern Academy of Sciences 92:101–167.

Van Doornik, D. A., R. S. Waples, M. C. Baird, P. Moran, and E. A. Berntson. 2011. Genetic monitoring reveals genetic stability within and among threatened Chinook Salmon populations in the Salmon River, Idaho. North American Journal of Fisheries Management 31:96–105.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotype errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Vandergast, A. G., A. J. Bohonak, D. B. Weissman, and R. N. Fisher. 2007. Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). Molecular Ecology 16:977–992.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555–561.

Waples, R. S., and C. Do. 2008. LDN_e: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.

Waples, R. S., and C. Do. 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.

Wells, A. W., J. S. Diana, and C. C. Swift. 1975. Survey of the freshwater fishes and their habitats in the coastal drainages of southern California. Final Report to the California Department of Fish and Game, Contract AB-26, Sacramento.