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ARTICLE

High Levels of Genetic Divergence Detected in Sacramento Perch Suggests Two Divergent Translocation Sources

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Abstract

Translocation has been used to conserve imperiled fishes and create new fisheries. One species for which translocation has played a significant role is the Sacramento Perch *Archoplites interruptus*. Extirpated from its native range, the Sacramento Perch has been introduced throughout California and Nevada through multiple translocation events, though historical records are incomplete. Recent assessments of eight previously uncharacterized Sacramento Perch populations have prompted reevaluation of range-wide population structure to inform a genetic management plan for long-term resiliency of this species. We examined Sacramento Perch genetic diversity and population structure across the current range of the species using 12 microsatellite markers. We analyzed samples from the eight uncharacterized populations and seven populations previously studied by Schwartz and May (2008). Bayesian clustering supported two distinct clusters of Sacramento Perch herein designated as A and B. Within these two clusters we detected hierarchical substructure, likely due to genetic drift after population founding. Genetic differentiation among populations within the same cluster was relatively low ($F_{ST} = 0.023–0.176$), while differentiation among populations from different clusters was higher ($F_{ST} = 0.190–0.320$). The existence of two strongly divergent genetic clusters in Sacramento Perch suggests two distinct translocation sources, and we recommend that these clusters be treated as genetic management

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units (GMUs). The B GMU populations had fairly low levels of genetic diversity relative to the A GMU populations. All populations showed evidence of past bottlenecks, and most had effective population sizes placing them at risk for inbreeding depression. Human-facilitated gene flow is recommended to prevent further genetic diversity loss. Due to uncertainty surrounding Sacramento Perch translocation history and strong levels of divergence between the two GMUs, translocations should be facilitated only between populations within the same GMU.

Fisheries managers use translocation as a tool to conserve imperiled species and create new fisheries (Williams et al. 1988; Minckley 1995; George et al. 2009). However, populations established through translocations are typically subject to genetic bottlenecks where the genetic diversity of the new population is a reduced representation of the source (Nei et al. 1975; Stockwell et al. 2009). Low genetic diversity of source populations, the number of available source populations, and the number of individuals available for translocation can influence the genetic diversity of the founded population (Drauch and Rhodes 2007; Dehaan et al. 2016). Further declines in genetic diversity can be accelerated by establishing small, isolated populations where the stochastic effects of genetic drift and the potential for inbreeding are increased (Meffe 1986). Reduced genetic diversity of founded populations is carried forward even as population size increases, potentially reducing its evolutionary potential and overall adaptive capacity (Soulé 1980; Reed and Frankham 2003). By examining the genetic structure and diversity of translocated populations, managers can develop plans to best conserve genetic diversity across populations and prioritize vulnerable populations for direct management actions.

The Sacramento Perch *Archoplites interruptus* is the only native centrarchid occurring in California and an example of a fish for which translocation has played a major role in the management and current distribution of the species. Historically, the Sacramento Perch was widely distributed in sloughs, slow-moving rivers, large lakes, and floodplain lakes throughout the Sacramento–San Joaquin drainage, the Pajaro and Salinas rivers, and Clear Lake (Moyle 2002). Due to overharvesting and dramatic hydrological alterations within its native range beginning during the California Gold Rush, the Sacramento Perch suffered declines in abundance (Rutter 1907; Moyle 2002). Populations were further imperiled by the introduction of nonnative centrarchids (Green Sunfish *Lepomis cyanellus* in 1891; Bluegill *Lepomis macrochirus* in 1908), which prey on Sacramento Perch eggs and larvae and competitively displace them from preferred habitat (Dill and Cordone 1997; Marchetti 1999; Moyle 2002). By 1976, the Sacramento Perch was extirpated from its native range (Acetiuno and Nicola 1976), and it is now considered a species of greatest conservation need by California Department of Fish and Wildlife (2015) and a fish species of critical concern (Moyle et al. 2015).

The popularity of the Sacramento Perch as a sport fish may have prevented its extinction. During the time in which native Sacramento Perch populations declined and were ultimately extirpated, several populations were established outside the species' native range, primarily for sportfishing purposes (McCarraher and Gregory 1970). As early as 1877, Sacramento Perch were translocated to lakes in Nevada. During the 1950s and 1960s, populations were established in reservoirs and lakes in California and Nevada, including Clear Lake Reservoir in California, which became a source for a natural invasion into the Klamath River system (Crain and Moyle 2011; Fuller 2020). Additional introductions were attempted in Arizona, Colorado, Nebraska, New Mexico, South Dakota, Texas, and Utah, but the species is considered extirpated in all these states except for Colorado and Utah (McCarraher and Gregory 1970; Crain and Moyle 2011). The legacy of these founding events is genetic bottlenecks and high differentiation between translocated populations (Schwartz and May 2008). Without active management, the species is at risk of continued losses of genetic diversity and adaptive capacity, thus making it less resilient to changing environmental conditions and stressors.

Recent assessment of eight genetically uncharacterized Sacramento Perch populations within California has prompted an expansion on previous work to evaluate genetic diversity and structure across the range of the species. This work is necessary to provide recommendations for genetic management to help ensure long-term resiliency of California's only native centrarchid. In this study, we aim to (1) describe the genetic diversity of the remnant populations, (2) examine how the various populations are related to each other through founding history, and (3) make recommendations for genetic management.

METHODS

Sample collection and DNA extraction.— Sacramento Perch were sampled in 2017 from eight previously uncharacterized locations within California by California Department of Fish and Wildlife biologists, with at least 40 individuals collected per location (Figure 1). Samples were collected by boat electrofishing at Gray Lodge Wildlife Area, West Valley Reservoir, Lake Almanor, and Benton Ponds. "Benton Ponds" refers to three ponds on private property near Benton, California. Through an agreement

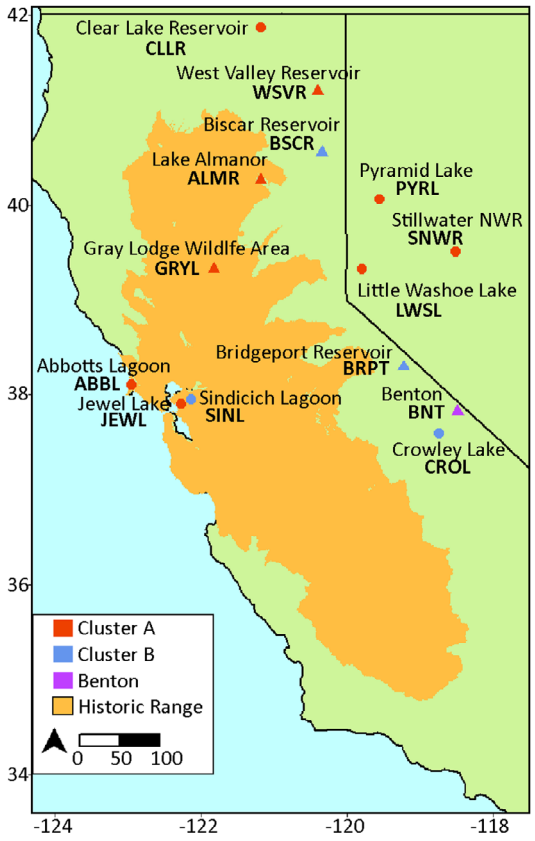

FIGURE 1. Range map of Sacramento Perch. The base layer of Sacramento Perch historic range is from NatureServe (2013). Sampling locations from Schwartz and May (2008) and this study are denoted by circles and triangles, respectively. Color indicates major genetic cluster.

with the landowner we were allowed to sample ponds 2, 3, and 4. Samples from Biscar Reservoir and Bridgeport were collected by hook and line due to lack of access and equipment malfunctions, respectively. A small piece of tissue was clipped from the pelvic fin of captured individuals. Fin clips were dried, stored in coin envelopes, and sent to the Genomic Variation Laboratory at University of California, Davis for extraction. A small subsample from each fin clip was used to extract DNA using the DNeasy Blood and Tissue Kit (QIAGEN). To reduce the risk of contamination, all extractions were done in a laboratory isolated from post-PCR products, and lab benchtops were bleached before and after tissue samples were handled.

Genotyping.—Samples were genotyped using 12 microsatellite loci optimized for use with Sacramento Perch: *AinA117, AinA2, AinA203, AinD119, AinA218, AinD106, AinA120, AinA216, AinA108, AinA6, AinA212,* and *AinD101* (Schwartz and May 2004). Microsatellite loci were combined into three multiplexes containing four loci each. Two microliters of PCR product were combined with 9.5 µl of highly deionized formamide and 0.5 µl of GeneScan 500 LIZ size standard (Thermo Fisher Scientific). Negative controls were included on each PCR plate to detect contamination. Fragment analysis was performed on an ABI PRISM 3730 DNA Analyzer (Thermo Fisher Scientific) and alleles were scored with STRand software (Toonen and Hughes 2001). Of the 353 samples analyzed, 19 samples were dropped from analysis because amplification failed at three or more loci. Also included in our study were 357 previously collected samples from Schwartz and May (2008) to assess the overall genetic structure of Sacramento Perch (Figure 1). All populations from Schwartz and May (2008) were included with the exception of Curved Pond, which had sufficient DNA remaining from <10 samples. Because genotypes from Schwartz and May (2008) were collected on a BaseStation DNA Fragment Analyzer (MJ Research) rather than a 3730 DNA Analyzer, we re-extracted and re-genotyped a subset of their samples on the 3730 DNA Analyzer. Samples were selected from each of the Schwartz and May (2008) populations so that every known allele was represented by at least one individual. Genotypes from Schwartz and May (2008) were then adjusted based on observed shifts in known genotypes so that both microsatellite data sets were compatible.

Genetic diversity.—Loci within putative populations were tested for deviation from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using GenAlEx version 6.503 and GENEPOP, respectively (Raymond and Rousset 1995; Peakall and Smouse 2006, 2012; Rousset 2008). Sequential Bonferroni corrections were used to account for multiple comparisons (Rice 1989). We examined genetic diversity within and among sampling locations by calculating the number of alleles, allelic richness, and expected and observed heterozygosity (H_e, H_o) in GenAlEx. Because small sample sizes can negatively bias genetic diversity estimates, we used rarefaction analysis in HP-Rare (Kalinowski 2005) to estimate allelic richness.

Relationships among populations.—Several approaches were used to examine how genetic diversity was partitioned among Sacramento Perch populations. First, we estimated pairwise F_{ST} values among putative populations according to Nei (1987) in the R package HIERFSTAT (Goudet 2005; R Core Team 2019). Significance was determined by 9,999 permutations in the program ADEGENET 2.1.0 package in R (Jombart 2008). We visualized the spatial structure of microsatellite data using discriminant analysis of principal components (DAPC) produced with ADEGENET. The DAPC optimizes the variance observed between groups while minimizing the variance within individuals to better visualize the relationship between samples. The optimal number of clusters was selected based on the lowest Bayesian information criterion value. We also constructed a neighbor-joining tree in APE (Paradis et al. 2004) based on Nei's genetic distance

to better visualize the relationships between various populations.

We used STRUCTURE version 2.3.4 (Pritchard et al. 2000) to evaluate population structure in our data set. Due to the potential for high levels of genetic similarity stemming from sequential stocking events from a single source population, we used the Hubisz et al. (2009) LOCPRIOR model that improves STRUCTURE's ability to detect weak population structure by using geographic sampling location as a prior, when informative. We also used the population admixture model with correlated allele frequencies. We began with exploratory runs consisting of 50,000 Markov chain–Monte Carlo iterations following a burn-in period of 5,000 iterations. We determined the likelihoods for $K = 1$ through $K = 16$, where K is the set number of populations. Ten replicates were conducted for each K. Longer runs consisting of 1,000,000 Markov chain–Monte Carlo iterations following a burn-in period of 100,000 iterations were then conducted. We calculated the likelihoods for a narrower range of $K = 1$ through $K = 10$, with 10 replicates for each K. We tested for the possibility of hierarchical substructure within each of the two genetic clusters initially detected with runs consisting of 1,000,000 Markov chain–Monte Carlo iterations following a burn-in period of 100,000 iterations. We calculated the likelihoods for $K = 1$ through $K = 9$ within each of the genetic clusters, with 10 replicates for each K. We determined the most likely K by examining plots of the mean likelihood value ln $\Pr(X|K)$ and calculating ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2012). The program CLUMPAK (Kopelman et al. 2015) was used to compile individual assignments across replicates, and we used a custom script implemented in the ggplot2 package in R to create bar plots to visualize results (Wickham 2016).

Population bottlenecks and effective population size.— We used four methods to test for genetic bottlenecks in each putative population. The program BOTTLENECK (Luikart and Cornuet 1998; Piry et al. 1999) was used to test for bottlenecks in sampled populations using the mode shift test, the sign test for excess heterozygosity, and the Wilcoxon's signed rank test for excess heterozygosity. Data were analyzed using the two-phase model for microsatellites with two different parameters sets as in Schwartz and May (2008). The first parameter set assumed that 95% of mutations were single step with a variance of 12 among multiple steps (recommended by authors of BOTTLENECK; Piry et al. 1999), and the second parameter set assumed 80% single step mutations with a variance of 50 among multiple steps (based on simulations of microsatellite evolution in sunfishes; Neff et al. 1999). One thousand replicates were performed to assess significance. The fourth method used for bottleneck testing was the M-ratio calculation. The M-ratio is based on the principle that microsatellite loci mutate through a stepwise process that creates a range of alleles in a population that differ in size by the repeat motif. In small populations, rare alleles are more likely to be lost through drift resulting in gaps in the allele distribution. Rapid reductions in population size due to bottlenecks create larger gaps in the allele distribution, and the M-ratio metric is sensitive to these gaps. A population is considered the have experienced a bottleneck if its M-value falls below a threshold of 0.67 (Garza and Williamson 2001). Four loci (*AinA117, AinA2, AinA218,* and *AinA120*) exhibited large gaps between groups of alleles for at least one of the populations and were therefore omitted from analyses because they did not conform to a stepwise mutation model (Schwartz and May 2008). Calculations of M-ratios were performed using the R package STRATAG (Archer et al. 2017).

To calculate the N_e of individual sample locations, we used the corrected linkage disequilibrium method (Waples and Do 2008) implemented in NeEstimator (Do et al. 2014), using a $P_{crit} = 0.02$ for populations where $N > 25$ samples to correct bias caused by rare alleles in larger populations. This method assumes random mating, isolation, selective neutrality of the markers used, no genetic structure within the populations, and discrete generations.

RESULTS

Genetic Diversity

Allele calls between the BaseStation and 3730 Genetic Analyzer differed by 1–3 bp depending on the locus. Using the re-extracted samples as references, Schwartz and May (2008) allele calls were adjusted to make their data set compatible with ours for further analysis. The number of alleles per locus ranged from 8 to 34, and the number of alleles per population ranged from 35 (Bridgeport and Crowley Lake) to 119 (Lake Almanor) out of the 194 total alleles detected in Sacramento Perch. Of these, 44 were private (i.e., unique to a single population). Gray Lodge Wildlife Area, Little Washoe Lake, Sindicich Lagoon, and West Valley Reservoir were the only populations that did not contain any private alleles. In the case of Gray Lodge Wildlife Area, this is likely because it was founded recently with individuals from Jewel Lake (Max Fish, unpublished data). Loci were polymorphic for all populations except for Crowley Lake, Sindicich Lagoon, and Stillwater National Wildlife Refuge, which were each monomorphic at one locus, and Bridgeport, which was monomorphic at two loci. Observed heterozygosity ranged from 0.24 to 0.79, whereas expected heterozygosity ranged from 0.29 to 0.79. Most populations had lower observed than expected heterozygosity except Clear Lake Reservoir, Jewel Lake, Little Washoe Lake, and West Valley Reservoir. Deviations in observed and expected heterozygosities were very slight, and none of these were found to be statistically significant (Table 1). A total of 34 locus pairs

deviated significantly from HWE expectations after a sequential Bonferroni correction ($P < 0.05$). Most populations had two or fewer loci deviating significantly from HWE. Abbotts Lagoon, Bridgeport, and Biscar Reservoir were found to have three, four, and five loci deviating significantly from HWE, respectively. There was no consistent pattern across populations or loci for significant deviations from HWE, so all loci were retained for subsequent analyses. Linkage disequilibrium was found between four pairs of loci after sequential Holm–Bonferroni correction (*P*-values from 0.00 to 0.03). Linkage disequilibrium was significant for *AinA108* and *AinA2* in Crowley Lake, *AinA108* and *AinD101* for Abbotts Lagoon, and *AinA203* and *AinA212* in Bridgeport, Sindicich Lagoon, and Pyramid Lake. The loci *AinA6* and *AinA212* showed significant linkage disequilibrium in 7 of the 15 populations (Abbotts Lagoon, Benton Ponds 2 and 3, Bridgeport, Biscar Reservoir, Crowley Lake, Gray Lodge Wildlife Area, and Pyramid Lake). This locus pair was noted to have significant linkage disequilibrium in five of the eight populations examined in Schwartz and May (2008). All populations showed linkage disequilibrium for two or more locus pairs except Lake Almanor, Clear Lake Reservoir, West Valley Reservoir, and Benton Pond 4, which had one. We expected linkage disequilibrium due to small population size and founder effects.

Relationships among Populations

All population pairs were genetically distinct, with pairwise F_{ST} values ranging from 0.023 to 0.320. All values were found to be statistically significant after sequential Holm–Bonferroni correction (adjusted *P*-value = 0.01) (Figure 2). Bridgeport had the most divergence from other populations, with pairwise F_{ST} values near or greater than 0.20 for 10 populations (Lake Almanor, Clear Lake Reservoir, Gray Lodge Wildlife Area, Jewel Lake, Little Washoe Lake, Pyramid Lake, Stillwater National Wildlife Refuge, West Valley Reservoir, Benton Ponds 2 and 3, and Benton Pond 4). Crowley Lake had the next greatest divergence from other populations, with pairwise F_{ST} values greater than 0.15 for 10 populations (Gray Lodge Wildlife Area, Jewel Lake, Lake Almanor, Clear Lake Reservoir, West Valley Reservoir, Little Washoe Lake, Pyramid Lake, Stillwater National Wildlife Refuge, Benton Ponds 2 and 3, and Benton Pond 4).

We identified seven distinct genetic clusters among Sacramento Perch samples using STRUCTURE based on the log probability of *K* (Figure 3). Delta *K* revealed a strong signal of genetic structure at $K = 2$, suggesting two major genetic clusters among Sacramento Perch samples with all but one population assigning to one of the two clusters (mean ln Pr $(X|K) = -28049.04$; Figure 4A). We refer to these two clusters as A and B. Cluster A comprised Abbotts Lagoon, Jewel Lake, Grey Lodge, West Valley Reservoir, Clear Lake Reservoir, Lake Almanor, Little Washoe Lake, Pyramid Lake, and Stillwater National Wildlife Refuge. Cluster B contained Biscar Reservoir, Sindicich Lagoon, Crowley, and Bridgeport. Only the Benton Ponds did not clearly assign to one of the clusters and instead showed evidence of admixture. Analyzing clusters A and B individually, we detected substructure within each cluster that was not revealed at $K = 7$ for the

TABLE 1. Number of Sacramento Perch sampled (*N*) from populations in California and Nevada, observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_r), private allelic richness (A_P), inbreeding coefficient (F_{IS}), and effective population size (N_{eLD} [calculated via the linkage disequilibrium method]; with 95% confidence limits [CL]) for Sacramento Perch.

Population		*N*	H_o	H_e	A_r	A_P	F_{IS}	N_{eLD} (95% CL)	
Abbotts Lagoon[a]	ABBL	132	0.65	0.66	5.44	0.43	0.01	337	(202–844)
Lake Almanor	ALMR	56	0.79	0.80	8.23	0.57	0.00	753	(250–∞)
Benton Pond 2 and 3	BNT23	49	0.55	0.62	4.37	0.08	0.03	222	(83–∞)
Benton Pond 4	BNT4	54	0.56	0.57	4.29	0.19	0.10	85	(50–195)
Bridgeport	BRPT	47	0.24	0.29	2.45	0.12	0.16	14	(8–25)
Biscar Reservoir	BSCR	36	0.42	0.43	3.02	0.08	0.03	9	(5–15)
Clear Lake Reservoir[a]	CLLR	27	0.73	0.73	6.7	0.25	0.00	569	(89–∞)
Crowley Lake[a]	CROL	53	0.42	0.43	2.72	0.06	0.03	22	(13–38)
Gray Lodge Wildlife Area	GRYL	42	0.68	0.68	5.04	0.02	0.01	21	(17–27)
Jewel Lake[a]	JEWL	24	0.72	0.72	5.94	0.27	–0.01	31	(21–52)
Little Washoe Lake[b]	LWSL	19	0.59	0.53	3.25	0.02	–0.10	∞	(∞–∞)
Pyramid Lake[a]	PYRL	45	0.65	0.69	5.93	0.61	0.02	65	(44–110)
Sindicich Lagoon[a]	SINL	24	0.41	0.41	3.09	0	0.01	13	(7–31)
Stillwater National Wildlife Refuge[a]	SNWR	33	0.52	0.57	4.04	0.1	0.08	75	(34–1102)
West Valley Reservoir	WSVR	50	0.77	0.75	7.1	0.11	–0.03	486	(183–∞)

[a]Populations from Schwartz and May (2008).
[b]Pyramid Lake was the original stocking location, but fish entered Little Washoe Lake via the Truckee River.

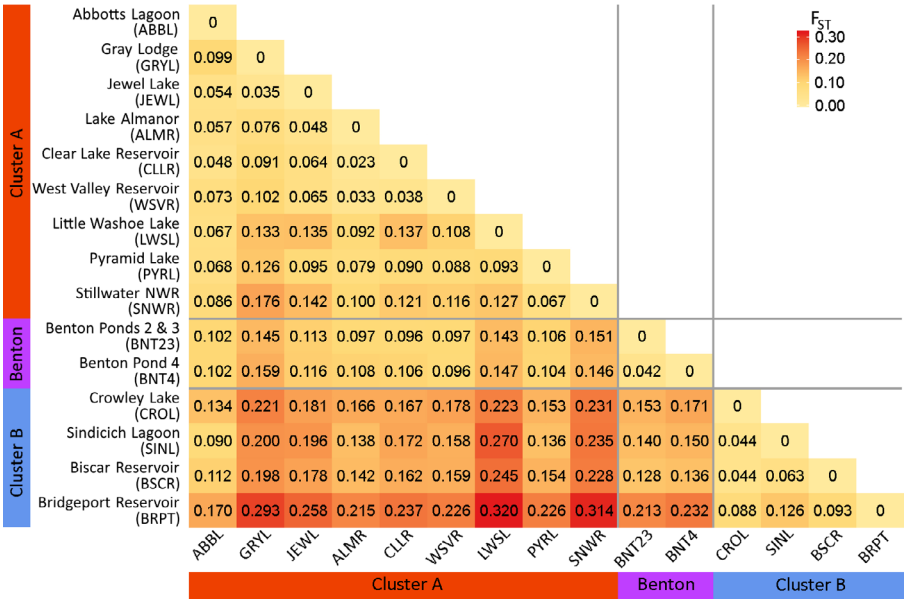

FIGURE 2. Pairwise F_{ST} heatmap for Sacramento Perch populations. Strong signals of differentiation were present between the A and B cluster populations, while the level of differentiation within the two major clusters was low. Values for F_{ST} were calculated following Nei (1987). All comparisons were found to be significant after sequential Holm–Bonferroni correction ($P = 0.01$).

whole data set. In these analyses, each cluster was analyzed with the Benton Pond populations included to determine how these admixed populations nested within the major clusters. For cluster B, the sampling locations indicated two or three subclusters where the Benton Ponds clustered independently of the other populations, and Bridgeport showed as an additional cluster at the higher K value (mean ln Pr(X|K) = –6,126.9 and –5,931.8, respectively; Figure 4A). Within cluster A, there was support for five subclusters (mean ln Pr(X|K) = –19,214.7). The Benton Ponds, Abbotts Lagoon, the Jewel Lake/Gray Lodge group, and the Nevada populations (Little Washoe Lake, Pyramid Lake, and Stillwater National Wildlife Refuge) retained their same assignment to separate clusters as seen at $K = 7$ for the whole data set. Unlike in the whole data set, the Lake Almanor, Clear Lake Reservoir, and West Valley Reservoir coalesced as one cluster instead of two. The spatial configuration of genetic relationships between populations in DAPC (Figure 4C) and the neighbor-joining tree (Figure 4B) also indicated two major clusters that were identical to those inferred from STRUCTURE results, with two subclusters within cluster B and four subclusters within cluster A. Benton Ponds were placed in between these two clusters.

Population Bottlenecks and Effective Population Size (N_e)

Depending on the method of bottleneck identification, between 4 and 12 populations in our data and those of Schwartz and May (2008) exhibited significant signatures of a bottleneck. Bottlenecks were identified for more populations with the Wilcoxon's signed rank test than any other test (Table 2; Schwartz and May 2008), possibly because the Wilcoxon's signed rank test is more sensitive than other tests when there are <20 loci (Piry et al. 1999). Lake Almanor, Benton Ponds 2 and 3, Crowley Lake, Gray Lodge Wildlife Area, Jewel Lake, Little Washoe Lake, and Stillwater National Wildlife Refuge showed bottleneck signatures under the standard parameter set with both Wilcoxon's signed rank and sign tests. Lake Almanor, Benton Ponds 2 and 3, Gray Lodge Wildlife Area, and Little Washoe Lake were the only populations that retained significant signatures for both the Wilcoxon's signed rank test and sign test when using centrarchid-specific microsatellite parameters (Table 2). We identified population bottlenecks in nine populations based on M-ratio tests: Benton Ponds 2 and 3, Benton Pond 4, Biscar Reservoir, Bridgeport, Crowley Lake, Gray Lodge Wildlife Area, Little Washoe Lake, Sindicich Lagoon, and Stillwater National Wildlife Refuge. Both Jewel Lake and Pyramid Lake were nearly significant for the M-ratio test.

Examination of the effective population sizes for both newly and previously sampled populations revealed a range of mean N_e values from 9.3 to 753.0 (Table 1). The 95% confidence limits for N_e tended to be wide. For Lake Almanor, Benton Ponds 2 and 3, and Clear Lake Reservoir, the upper confidence limit was infinity, indicating that we had low power to estimate N_e using the LDNe method (Waples and Do 2008).

DISCUSSION

Uncertainty about the methods and source populations used in translocation efforts muddies our understanding of

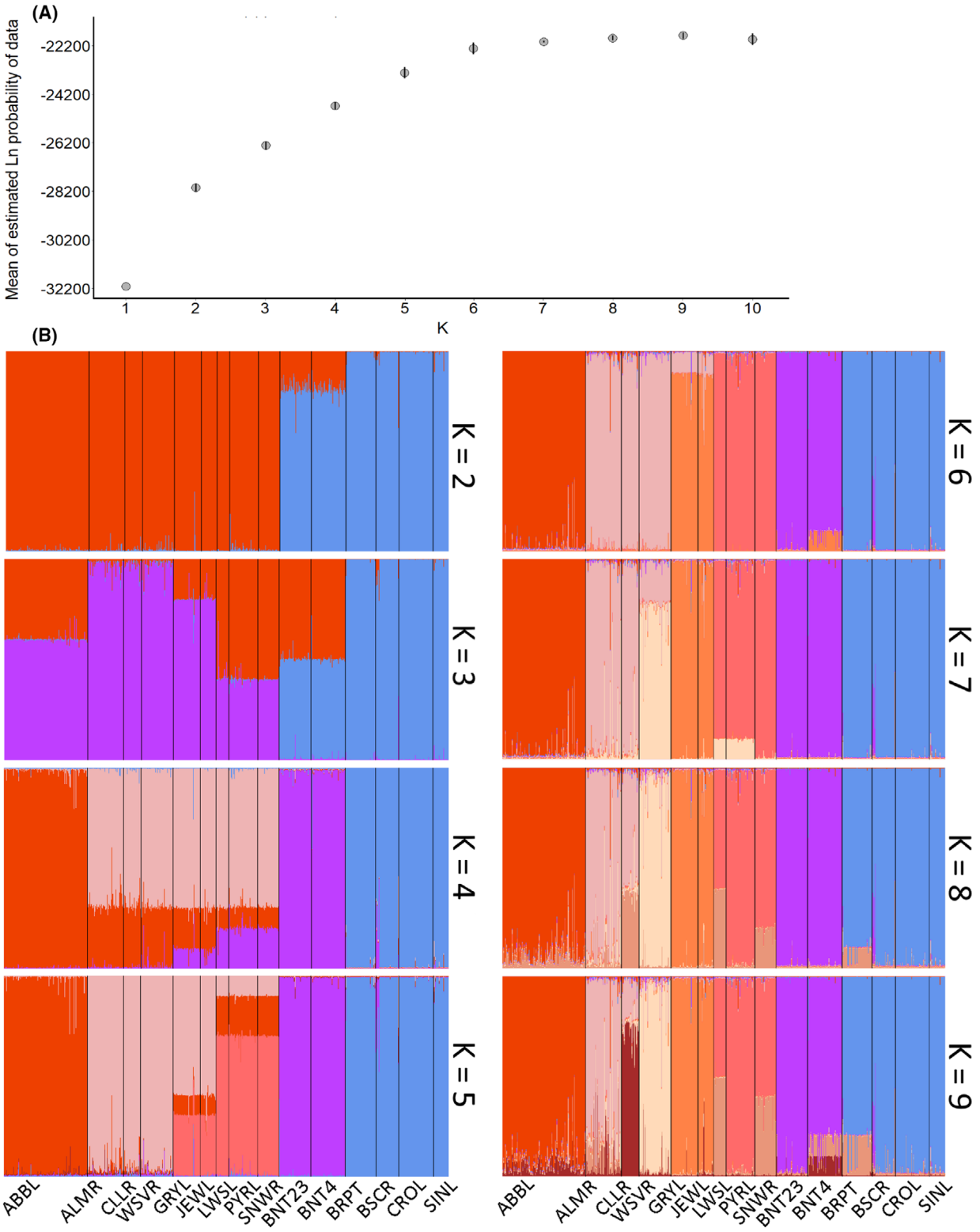

FIGURE 3. Resolution of genetic relationships between Sacramento Perch populations with **(A)** a plot of the mean estimated log probability of the data for $K = 1$ through $K = 10$ and **(B)** results of the STRUCTURE analysis of $K = 2$–9. The STRUCTURE analysis revealed that $K = 7$ was most likely as no additional populations resolved at higher clusters. Individuals are represented by bars in each plot and the proportion of colors within each bar represents proportional ancestry in each genetic cluster. See Table 1 for population codes.

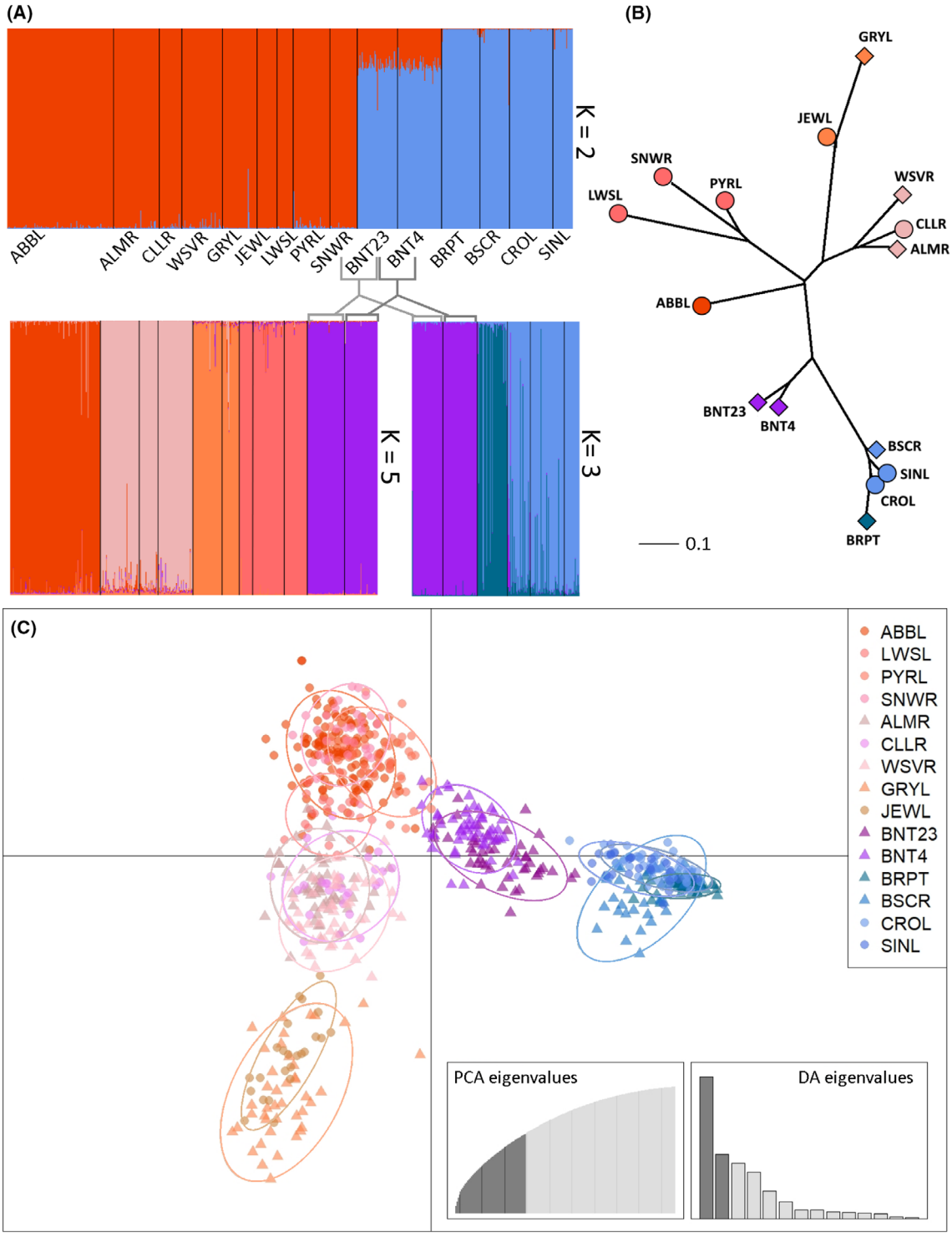

FIGURE 4. Genetic relationships among Sacramento Perch populations within major clusters. See Table 1 for population codes. **(A)** The STRUCTURE analysis indicated that most Sacramento Perch populations belong to one of two major genetic clusters. We refer to the two groups as cluster A (red) and cluster B (blue). Hierarchical structure was present within these two clusters, with robust support for three genetic groupings ($K = 3$) in cluster B, including Benton (cool colors) and five genetic groupings in cluster A including Benton ($K = 5$) (warm colors). **(B)** A neighbor-joining tree of Sacramento Perch populations was constructed based on Nei's genetic distances. **(C)** Discriminant analysis (DA) of principal components of Sacramento Perch supported the distinction of clusters A (warm colors) and B (cool colors) with hierarchical structure within each. Circles are populations sampled by Schwartz and May (2008), while triangles are newly sampled populations. See Table 1 for population codes.

TABLE 2. Bottleneck tests for Sacramento Perch populations examining *M*-statistic (*M*), mode shift test (*Mode*), sign test (*S*), and Wilcoxon's signed rank test (*W*) under two different parameter sets: Parameter set 1 (default model), and Parameter set 2 (centrarchid-specific model). Bold values indicate significant bottleneck tests. Population codes are defined in Table 1.

		Parameter set 1			Parameter set 2		
Population	*M*	*Mode*	*S* (*P*-value)	*W*	*Mode*	*S* (*P*-value)	*W*
ABBL	0.80	Normal	7.04 (0.070)	**(0.026)**	Normal	7.11 (0.417)	(0.117)
ALMR	0.85	Normal	**7.16 (0.019)**	**(0.017)**	Normal	**7.16 (0.019)**	**(0.017)**
BNT23	**0.57**	Normal	**6.84 (0.012)**	**(0.017)**	Normal	**6.91 (0.013)**	**(0.017)**
BNT4	**0.59**	Normal	6.73 (0.150)	**(0.039)**	Normal	6.82 (0.163)	(0.076)
BRPT	**0.64**	Normal	5.09 (0.353)	(0.652)	Normal	5.14 (0.341)	(0.688)
BSCR	**0.59**	Normal	6.30 (0.243)	(0.212)	Normal	6.43 (0.268)	(0.235)
CLLR	0.82	Normal	7.14 (0.215)	**(0.002)**	Normal	7.14 (0.215)	(0.055)
CROL	**0.57**	Normal	**5.48 (0.030)**	**(0.005)**	Normal	5.55 (0.117)	**(0.008)**
GRYL	**0.61**	Normal	**6.95 (0.001)**	**(1.2×10^{-4})**	Normal	**6.98 (0.014)**	**(2.4×10^{-4})**
JEWL	0.69	Normal	**7.08 (0.002)**	**(1.2×10^{-4})**	Normal	7.11 (0.076)	**(0.001)**
LWSL	**0.59**	Normal	**6.37 (0.005)**	**(0.003)**	Normal	**6.43 (0.006)**	**(0.017)**
PYRL	0.73	Normal	**6.95 (0.014)**	**(0.001)**	Normal	6.93 (0.061)	**(0.006)**
SINL	**0.52**	Normal	5.64 (0.302)	(0.103)	Normal	5.70 (0.314)	(0.160)
SNWR	**0.65**	Normal	**6.33 (0.021)**	**(0.006)**	Normal	6.41 (0.098)	**(0.042)**
WSVR	0.85	Normal	7.18 (0.081)	**(0.021)**	Normal	7.14 (0.078)	**(0.021)**

how Sacramento Perch remnant populations were founded. Most translocated populations with historical records were sourced from the Sacramento River or nearby ponds containing individuals sourced from surrounding water bodies, including the Sacramento River. Based on this knowledge, we expected that all extant populations of Sacramento Perch would show low differentiation aside from minor changes in allele frequencies due to drift in isolation. Instead, we observed significant levels of genetic differentiation across the range of Sacramento Perch, with populations assigning to one of two genetic clusters and with groups of populations clustering differently during hierarchical analyses.

Differentiation between the A and B clusters likely represents stocking from two isolated sources, which were either geographically distant but in the same drainage or from separate drainages. Populations from the same drainage that were isolated by distance would have experienced decreased gene flow and increased drift, leading to genetic differentiation among populations. Intermediate populations in the Sacramento–San Joaquin drainage could have been lost as the species was extirpated from its range, resulting in the appearance of two genetically distinct lineages. Alternatively, the two clusters could represent lineages from different drainages. Sacramento Perch were described outside the Sacramento–San Joaquin drainage in Clear Lake and the Salinas and Pajaro rivers (Aceituno and Nicola 1976; Gobalet 1993). Although gene flow likely occurred between Clear Lake and the Sacramento River until the construction of the Cache Creek Dam in 1915, the Salinas and Pajaro rivers have been isolated from the Sacramento–San Joaquin Basin for more than 600,000 years (Martin and Emery 1967; Normark 1999; Thompson et al. 2013). If sourced from the Salinas or Pajaro rivers, the B cluster could represent genetic differentiation resulting from isolation on a geologic time scale. Thus, we propose that the A and B genetic clusters should be managed as separate genetic management units (GMUs).

Relationships among Populations

Within the two major clusters, STRUCTURE, DAPC, and the neighbor-joining tree all supported the presence of seven subclusters. Nearly all populations nested within one of the two major clusters, the A and B GMUs. This suggests that populations within the same GMU likely originated from the same source population. The existence of hierarchical structure despite common ancestry suggests that founder effects and significant genetic drift occurred within small, isolated Sacramento Perch populations.

The few translocation records that exist for Sacramento Perch are for A GMU populations and support the hierarchical structure observed. The Nevada populations (Little Washoe Lake, Pyramid Lake, and Stillwater National Wildlife Refuge) were all stocked with descendants from a translocation of Sacramento River fish to Washoe Lake in 1877. Another subcluster of populations (Clear Lake Reservoir, Lake Almanor, and West Valley Reservoir) were stocked with individuals from the Central Valley

Fish Hatchery, sourced from various ponds around the Sacramento Valley. Interestingly, Crowley Lake, which was likely established during the same period as the Central Valley Fish Hatchery translocations, clustered with the B GMU.

Only the Benton Pond populations did not definitively assign to a GMU, showing admixed ancestry from both the A and B GMUs. As with the B GMU populations, there is no record of the stocking history for the Benton Pond populations, which exist on private property. Genetic analyses did not identify possible sources used for translocation into Benton Ponds. Low pairwise F_{ST} and early clustering with populations derived from the Sacramento River suggest shared ancestry with at least one A GMU population. There was no support for a candidate source in the B GMU. Further refinement of Benton Pond ancestry would require markers with greater power than the 12 microsatellite loci used for this study.

Genetic Diversity

Hatchery propagation and stocking began after the species had already declined, which would have lowered the standing genetic variation available to establish populations (Rutter 1907; Crain and Moyle 2011). Extant Sacramento Perch populations exhibit a range of genetic diversity levels with mean heterozygosities similar to those previously described by Schwartz and May (2008) and for other centrarchids (Coughlin et al. 2003; Stepien et al. 2007). Differences between populations in the time of founding, number of founders, diversity of the source populations, and number of stocking events can explain differences in allelic richness and heterozygosity observed in this study. Populations in the A GMU tended to show greater genetic diversity and less linkage disequilibrium than those in the B GMU. Lower genetic diversity in B GMU populations could be due to sequential founding events from Crowley Lake with low numbers of individuals (LeCorre and Kremer 1998; Pruett and Winker 2005).

Population Bottlenecks and Effective Population Size

Populations established through translocation often exhibit reduced genetic diversity when compared to the original source population (Stockwell et al. 2009; Finger et al. 2013). Translocating a large number of individuals can maximize the probability that source genetic diversity will be retained in the new population (Dehaan et al. 2016). Despite best efforts, not all individuals will contribute equally to subsequent generations, so original stocking numbers will not correspond to the estimated effective population size.

The N_e varied widely among Sacramento Perch populations, with most falling below the minimum recommended value to prevent further genetic diversity loss from inbreeding and drift. Current conservation recommendations are to establish populations with an N_e of 100 or greater to avoid inbreeding depression, and 1,000 or higher to maintain evolutionary potential (Frankham et al. 2014). No Sacramento Perch populations meet the threshold needed to retain evolutionary potential, and only five are above the threshold to prevent inbreeding depression. However, we acknowledge that our estimates may be somewhat downwardly biased because our samples include individuals from multiple generations (Waples et al. 2014). Apart from Benton Ponds 2 and 3, which are of admixed ancestry, populations with $N_e > 100$ all belong to the A GMU. Of these, only Abbotts Lagoon was not sourced from the Sacramento River.

Bottlenecks in Sacramento Perch populations could have occurred across various time points. Causes of genetic diversity loss include the initial decline of the species, founder effects from translocation, and stochastic environmental events such as severe drought. During drought, Sacramento Perch populations isolated in small ponds are threatened with desiccation, while storage reservoirs are subject to water level fluctuations. Mismatches in the time of nesting and dropping water levels due to increased water demands can result in stranded nests and low breeding success, which could cause genetic bottlenecks (Crain and Moyle 2011).

The three methods we used to identify population bottlenecks are best suited to detecting recent bottlenecks. The heterozygosity excess methods can detect bottlenecks that have occurred <4 N_e generations prior, while the mode shift test best detects bottlenecks occurring within several dozen generations (Luikart and Cornuet 1998; Luikart et al. 1998). Most of the remnant Sacramento Perch populations showed significant signatures of recent bottlenecks, as would be expected given that they experienced one or more founding events. Bottlenecks in Sacramento Perch populations occurring >80 years ago may not be detected by these methods, such as for the initial translocation of Sacramento Perch into Nevada in 1877. The *M*-ratio test (Garza and Williamson 2001) is a better alternative for detecting historical or more protracted bottlenecks, such as the initial population contraction. Historical bottleneck signatures were detected with the *M*-ratio test in all but six populations. Jewel and Pyramid lakes were only marginally above the significance threshold for being considered bottlenecked under the *M*-ratio test (Garza and Williamson 2001). Only Abbotts Lagoon and the populations sourced from the Sacramento River did not carry historical bottleneck signatures. These populations may have been founded earlier or from more genetically diverse sources than the rest of the populations.

Gray Lodge Wildlife Area provides an example of the additive impacts of sequential translocations. In 2014, Sacramento Perch were translocated from Jewel Lake to Gray Lodge Wildlife Area to "rescue" the population. Jewel Lake showed signatures of a recent bottleneck and a

marginally significant historical bottleneck signature. The Gray Lodge Wildlife Area population showed significant signals of recent and historical bottlenecks and lower genetic diversity than Jewel Lake. The stronger historical bottleneck signature in Gray Lodge Wildlife Area relative to Jewel Lake could reflect additive impacts of drift in Jewel Lake and a subsequent founder effect. The loss of two rare, private alleles over the 10 years between the sampling of Jewel Lake for Schwartz and May (2008) and the rescue translocation highlights the risk of continued genetic diversity loss in remnant populations.

Management Implications

Translocating individuals to establish new populations for recreational or commercial fisheries can facilitate conservation by decreasing the probability that stochastic events in one location could result in the total loss of the species (Minckley 1995). Simply establishing new populations on an ad hoc basis is not enough to ensure survival of an imperiled species. Careful planning is required to minimize reductions in genetic diversity when establishing new populations via translocation, in addition to human-mediated gene flow among populations (George et al. 2009). Persistence of small, isolated Sacramento Perch populations will require active management to slow genetic diversity loss caused by genetic drift and inbreeding.

While not considered demographically rare, most Sacramento Perch populations have low genetic diversity and low effective population sizes. Supplementation of these populations will reduce further genetic diversity loss and the risk of inbreeding depression, particularly if multiple sources with high levels of genetic diversity are used (Falk et al. 2001). Lake Almanor, Clear Lake and West Valley reservoirs are the most suitable sources for supplementation of genetically depauperate populations. The Nevada populations and others established by out-of-state translocations from Pyramid Lake share a similar caveat as the populations founded from the Central Valley Fish Hatchery: all belong to the A GMU and overrepresent Sacramento River ancestry. The B GMU populations that meet genetic diversity criteria are Benton Ponds 2 and 3, and they, along with Benton Pond 4, are of admixed ancestry and found on private land.

Supplementation with sources from within a GMU is recommended until we better understand the cause of genetic differentiation between the two major lineages. The risk of outbreeding depression is lower for populations located in similar environments that have experienced genetic exchange within the past 500 years (Frankham et al. 2011), but if A and B GMUs originate from different drainages and represent significantly different evolutionary legacies, outbreeding depression could occur if individuals from A and B GMUs are mixed. If the Benton Ponds truly represent admixture of fish from the two major lineages, they provide an excellent opportunity to observe effects of mixing GMUs.

Management of Sacramento Perch should not rely solely on translocations between the remnant populations. A conservation hatchery can help facilitate supplementation of current populations without depleting sources and can also be used as the source for individuals in other California Department of Fish and Wildlife projects involving Sacramento Perch. As well as being a fish of interest for recreational fisheries, as the state's only native centrarchid, this species has potential as a California native alternative for *Tilapia* aquaculture and *Gambusia* mosquito control, highlighting the diverse benefits of native species management (P. Moyle, University of California–Davis, personal communication). In addition to providing individuals for genetic rescue, a conservation hatchery program would provide a source for establishing additional refuge populations, which would further reduce the loss of genetic diversity and minimizes the risk of extinction.

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