# Genetic Evaluation of Isolated Populations for Use in Reintroductions Reveals Significant Genetic Bottlenecks in Potential Stocks of Sacramento Perch

**Article** *in* Transactions of the American Fisheries Society · November 2008

DOI: 10.1577/T07-241.1

CITATIONS
6

READS
82

**2 authors**, including:

Rachel Schwartz
Arizona State University
**20** PUBLICATIONS **391** CITATIONS

SEE PROFILE

*Transactions of the American Fisheries Society* 137:1764–1777, 2008

DOI: 10.1577/T07-241.1

[Article]

# Genetic Evaluation of Isolated Populations for Use in Reintroductions Reveals Significant Genetic Bottlenecks in Potential Stocks of Sacramento Perch

RACHEL S. SCHWARTZ* AND BERNIE MAY

*Department of Animal Science, University of California–Davis, Meyer Hall, One Shields Avenue, Davis, California 95616, USA*

*Abstract.*—New populations of threatened species are often established as a conservation measure. However, if only a few individuals contribute to subsequent generations, these populations may have limited genetic diversity. Such genetic bottlenecks can result in inbreeding depression, reduced fitness, and even extirpation of populations. Eight isolated populations of Sacramento perch *Archoplites interruptus* established through anthropogenic translocations were examined for evidence of genetic bottlenecks. Sacramento perch are endemic to two regions of California but have been entirely extirpated from their native range; the remaining populations are essential for conservation of the species. Using 12 microsatellite DNA loci, we determined that genetic bottlenecks occurred in six of the populations. Allelic richness, richness of private alleles, and effective population size differed significantly among populations. Strong differentiation among the extant populations probably resulted from differences in the sources used to establish the populations and from genetic drift due to the small population sizes. These results indicate that genetic bottlenecks are frequent when new, isolated populations of a species are established. Although these extant populations have persisted despite bottlenecks, future Sacramento perch populations should be established by drawing from the most diverse of the current populations and should be monitored with genetic markers to evaluate diversity and the possible need for further stocking. We combine three measures of genetic diversity (allelic richness, private allelic richness, and effective population size) to recommend potential source populations.

Conservation efforts often focus on increasing a species' likelihood of survival by establishing new populations in restored habitat. However, populations are sometimes established with small numbers of individuals if few fish are available or if stocking costs are prohibitive. Alternatively, even when a large number of individuals is used to establish a population, only a few individuals may contribute to future generations. Genetic bottlenecks can result from either situation, greatly reducing the genetic diversity of a new population relative to that of the source population (e.g., Robichaux et al. 1997; Maudet et al. 2002). Even when populations recover from demographic declines, they retain limited genetic diversity (e.g., Maudet et al. 2002; DeYoung et al. 2003) and therefore may be subject to detrimental effects of environmental changes that would be expected only in smaller populations.

Genetic diversity plays an important role in population survival by maintaining the species' potential to adapt to stochastic events and thereby reducing the risk of extinction (Reed and Frankham 2003). Through inbreeding and the expression of deleterious alleles, loss of genetic diversity can reduce the fitness of a population and its ability to survive stochastic events. Franklin (1980) suggested that an inbreeding rate of 1% is acceptable to minimize inbreeding depression and to maintain a population's ability to adapt in the short term; attainment of the acceptable rate of inbreeding requires a minimum effective population size ($N_e$) of 50. To maintain the ability to adapt in the long term, a population should not lose more additive genetic variance through drift than is gained by mutation; Franklin (1980) estimated that a population with an $N_e$ greater than 500 will meet this requirement. However, Lande (1995) argued that an $N_e$ of 5,000 may be necessary to maintain two-thirds of additive genetic variance, given quasineutral mutations.

Holsinger and Vitt (1997) particularly emphasized the significance of changes in the genetic makeup of captive broodstocks; such changes create the potential for inbreeding depression, loss of diversity, and local adaptation. We believe that wild stocks established through translocations could exhibit similar problems as a result of genetic bottlenecks, which could prove detrimental if the established populations are themselves used as sources for further stocking. Loss of genetic diversity due to isolation is of particular concern in aquatic species because aquatic habitats can act as islands that are completely isolated by intervening land and because the survival of aquatic

* Corresponding author: rachel.schwartz@colostate.edu

Received November 12, 2007; accepted June 4, 2008
Published online December 15, 2008

species depends on a number of environmental variables (e.g., dissolved oxygen, temperature, salinity, and alkalinity). Although fish are frequently moved between lakes and streams, the possibility that these movements result in genetic bottlenecks is rarely addressed.

To evaluate whether isolated populations of stocked fish frequently experience genetic bottlenecks, we studied genetic variation in the Sacramento perch *Archoplites interruptus*, a centrarchid fish that is endemic to California. The Sacramento perch was once the dominant piscivorous fish in the Central Valley of California and therefore was a key component of Central Valley aquatic ecosystems (Moyle 2002). The native range of the Sacramento perch was limited to the Sacramento–San Joaquin River watershed, the Pajaro and Salinas River drainages, Clear Lake (Lake County), and Alameda and Coyote creeks (Figure 1). This species was once so abundant that native people in California relied on it for food (Gobalet et al. 2004), and it was fished commercially in the late 19th century, reportedly yielding nearly 0.5 million lb (226,796 kg) on the San Francisco fish market in a single year (Skinner 1962). However, no known populations of Sacramento perch remain in the species' native range (P. Crain, University of California–Davis, personal communication), although the dates of population extirpations are generally unknown due to a lack of routine surveys for this species.

Sacramento perch have been described as having a poor ability to compete with other centrarchids for food and habitat and as needing coldwater habitat such as would be provided by a naturally flowing Sacramento River system (Marchetti 1999). Consequently, hypothesized reasons for the extirpation of Sacramento perch include competition and predation from nonnative centrarchids (e.g., bluegill *Lepomis macrochirus*), anthropogenic habitat changes (e.g., altered flow regimes and reduced spawning and rearing habitat), and overfishing (Moyle 2002; P. Crain, C. Woodley, University of California–Davis, and T. Carpenter, Archaeometrics Inc., Davis, California, personal communication). Sacramento perch have been targeted for recovery in the Delta Native Fishes Recovery Plan (Moyle et al. 1996), listed as a species of special concern by the California Department of Fish and Game, and classified as an at-risk species (priority group 2) by the 2001 Ecosystem Restoration Program of CALFED, a cooperative federal–state program that is overseen by the California Bay–Delta Authority (CBDA). Reintroductions of Sacramento perch are currently being planned; locations with coldwater habitat and few nonnative competitors have been selected to minimize competitive exclusion and maximize survival of introduced populations.


FIGURE 1.—Map of the native range of Sacramento perch in California (small italic text) and the locations of known extant populations in California and Nevada (large upright text).

Sacramento perch have been translocated numerous times, and several extant populations outside of the species' native range have resulted. Because no populations of Sacramento perch remain in the San Francisco Bay–Delta area (part of the native range), reintroduction of the Sacramento perch is an essential part of the restoration of this ecosystem. Additionally, because many of the extant populations are located in environments managed for human use, such as reservoirs, the long-term stability of these populations is questionable. The Sacramento perch would probably be listed as an endangered species if populations were not established outside the native range; because of its limited local abundance, this species may be particularly prone to extinction (Pyron 1999). We hypothesized that given differences in founding numbers and original diversity among the extant populations, some populations would be more suitable than others as sources for reintroduction.

Eight populations of Sacramento perch are potential sources for either direct reintroduction or further propagation in fish hatcheries. Six of the populations are located in California: Abbotts Lagoon at Point Reyes National Seashore; Jewel Lake in Tilden Park, Berkeley; Sindicich Lagoon in Briones Park, Martinez; Curved Pond on the University of California–Davis campus; Clear Lake Reservoir in Modoc County; and Crowley Lake north of Bishop. Two populations also

TABLE 1.—Sample locations, sample sizes (*N*), sampling years, source (if known), original population size, and year of establishment for eight extant populations of Sacramento perch in California (CA) and Nevada (NV). These populations were established outside of the historic native range, from which the species is now extirpated.

| Population | Location | *N* | Sample year | Population origin | Original size | Origin year |
|---|---|---|---|---|---|---|
| Sindicich Lagoon | Martinez, California | 33 | 2003 | Unknown | Unknown | Unknown |
| Stillwater National Wildlife Refuge | Fallon, Nevada | 35 | 2003 | Unknown | Unknown | Unknown |
| Clear Lake Reservoir | Modoc County, California | 29 | 2003 | Clear Lake via Elk Grove Hatchery[a] | 669[b] | 1950s[b] |
| Abbotts Lagoon | Pt. Reyes, California | 48, 51, 40 | 2001, 2002, 2005 | Unknown | Unknown | Unknown |
| Pyramid Lake | Reno, Nevada | 39 | 2004 | Washoe Lake via Truckee River[c] | <700 | After 1877 |
| Crowley Lake | Mono County, California | 19, 53 | 2004, 2005 | Unknown | Small | 1960s[b] |
| Jewel Lake | Berkeley, California | 14, 24 | 2004, 2005 | Unknown | Unknown | 1964 |
| Curved Pond | Davis, California | 41 | 2004 | Unknown | 67[b] | Recent |

[a] Clear Lake Reservoir may have been stocked with hatchery fish of Clear Lake stock.
[b] Rough estimate with high uncertainty.
[c] Fish from the Sacramento River were stocked into Washoe Lake. Some of these fish then entered the Truckee River and eventually reached Pyramid Lake.

exist in Nevada: Pyramid Lake near Reno and Stillwater National Wildlife Refuge (NWR) near Fallon (Figure 1). Only a few of these populations are of known origin (Table 1). A lake upstream of Jewel Lake was stocked in 1964 (P. Alexander, East Bay Regional Park District, Oakland, California, personal communication). Curved Pond was recently stocked with 37 subadults and was later supplemented with 30 individuals from a different population (P. Crain, personal communication). Clear Lake Reservoir was reportedly stocked with 669 individuals during the middle of the 20th century (P. Crain, personal communication). The Pyramid Lake population was probably established by fish moving through the Truckee River from Washoe Lake, where 700 individuals from the Sacramento River were stocked in 1877 (M. Warren, Nevada Department of Wildlife, personal communication). In contrast, the population in Crowley Lake probably originated from the private, illegal stocking of just a few individuals; Sacramento perch were first caught in Crowley Lake in 1964 (P. Pister, California Department of Fish and Game, personal communication). This information is summarized in Table 1.

In this study, we used variation in microsatellite DNA loci to (1) determine levels of genetic variation in the eight extant populations, (2) evaluate the genetic history of the populations, and (3) determine the extent to which the populations are genetically distinct from one another. We used these results to demonstrate the importance of considering genetic variation in reintroductions, to suggest strategies for reintroducing Sacramento perch to the native range, and to propose potential source populations for reintroduction projects. This study also serves as a baseline for future analysis of reestablished populations and can be used to identify differential success of individuals from different sources and to determine the number of individuals contributing to future generations.

## Methods

*Sample collection*.—Sacramento perch were sampled from the eight populations in California and Nevada. Fish were caught by electrofishing, hook and line, gillnetting, or seining. For each fish, a small piece of tissue was clipped from the anal fin or a few scales were removed; fish were released unharmed whenever possible. Tissue samples from at least 29 individuals were collected from each population; for three populations, tissue samples were collected in multiple years to ensure that variability among years was less than that among populations (Table 1).

*Extraction of DNA and microsatellite genotyping*.—The DNA was extracted from fin clips stored in 95% ethanol (Stillwater NWR, Clear Lake Reservoir, Pyramid Lake, Crowley Lake, Jewel Lake, and Curved Pond), fin clips dried in coin envelopes (Sindicich Lagoon), or scales dried in coin envelopes (Abbotts Lagoon). Extractions were conducted by using either Promega Genomic DNA purification system or Qiagen DNeasy extraction kits. Individuals were genotyped for 12 tetranucleotide microsatellite loci: *AinA117*, *AinA2*, *AinA203*, *AinD119*, *AinA218*, *AinD106*, *AinA120*, *AinA216*, *AinA108*, *AinA6*, *AinA212*, and *AinD101* (Schwartz and May 2004). Loci were selected on the basis of repeated successful amplification in multiple populations. Polymerase chain reaction (PCR) was performed in a 10-μL total volume consisting of 5–10

ng of DNA, 1× Promega DNA polymerase Mg-free buffer, 2 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (Promega), 0.5 μM of each primer, and 0.375 units of Promega *Taq* DNA polymerase. Each 5' primer was fluorescently labeled with FAM, VIC, or NED fluorophores (Applied Biosystems, Inc. [ABI]). Amplification was performed with an MJ Research PTC-100 or Dyad thermal cycler as follows: 94°C for 150 s; 25 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and a final extension at 60°C for 30 min. Two or three PCR products with different fluorescent labels (1 μL each) were mixed with 2 μL of water, 1.45 μL of 100% de-ionized formamide (Sigma), 0.5 μL of blue dextran loading dye (ABI), and 0.05 μL of ABI GeneScan 400HD (ROX) internal size standard. Samples were denatured at 95°C for 210 s and were chilled on ice. From the PCR product mixture, 1–2 μL were loaded on a 5.5% denaturing polyacrylamide gel in 1× tris–borate–EDTA in a Basestation DNA fragment analyzer (MJ Research). Gels were prerun at 1,900 V for 2 min, samples were loaded for 30 s at 4,000 V, and 5,600 scans were made at 2,600 V. Fragment sizes were scored for each locus by using Cartographer software (MJ Research). Eleven samples were excluded from analyses because amplification failed for three or more loci. No single locus appeared to have a higher failure rate than any other locus.

*General statistics*.—Loci within populations were tested for deviation from expected Hardy–Weinberg frequencies using the exact test of Guo and Thompson (1992) with a Markov-chain Monte Carlo (MCMC) estimator of the probability that the observed sample was taken from a population in Hardy–Weinberg equilibrium. This analysis was implemented in GENEPOP version 3.4 (updated from Raymond and Rousset 1995). Markov-chain parameters were set at 1,000 dememorization steps, 100 batches, and 1,000 iterations/batch. Loci within populations were tested for deviation from linkage equilibrium by use of an MCMC estimate for Fisher's exact test in GENEPOP (parameters were identical to those described for the preceding analysis). The α value for each statistical test was adjusted by a sequential Bonferroni correction (Rice 1989). We used GENEPOP to calculate allele frequencies and observed and expected heterozygosities. Differences between observed and expected heterozygosities within populations and differences in observed heterozygosity between populations were determined by using paired *t*-tests.

*Relationships between populations*.—Differentiation between pairs of populations was calculated in GENETIX (Belkhir et al. 2004) based on Cavalli-Sforza and Edwards (1967) chord distances ($D_{CSE}$) and was drawn as a neighbor-joining dendrogram in Molecular Evolutionary Genetics Analysis version 3.1 (Kumar et al. 2004). The $D_{CSE}$ was chosen over other measures, such as Nei's (1972) genetic distance, because the $D_{CSE}$ model assumes no mutation (i.e., assumes that changes in gene frequency depend solely on genetic drift) and allows for fluctuating $N_e$; this model is most compatible with the known history in which (1) only a few individuals were used to found current populations and (2) recent introductions were responsible for differentiation between the populations. Pairwise and overall values of the genetic differentiation index ($F_{ST}$; Wright 1965) were estimated as Weir and Cockerham's (1984) θ in FSTAT (Goudet 2001). $F_{ST}$ is a measure of reduced heterozygosity in subpopulations relative to heterozygosity in the overall population, thus indicating population structure. Calculation of $F_{ST}$ assumes the infinite-alleles model, whereas other genetic differentiation measures such as $R_{ST}$ (Slatkin 1995) assume a stepwise mutation model. Neither $F_{ST}$ nor $R_{ST}$ produces accurate results if the loci conform to a two-phase model, as was suggested by Neff et al. (1999) for microsatellite DNA in sunfishes. However, we chose to use $F_{ST}$ because of the disjunct distribution of alleles in most loci and populations.

*Tests for population bottlenecks*.—Given the likelihood that extant populations were established from a small number of individuals (P. Moyle, University of California–Davis, personal communication), we tested each sampled population for genetic bottlenecks by using (1) the *M*-test (Garza and Williamson 2001); (2) a test for mode-shifted distributions (Luikart et al. 1998); (3) the sign test for excess heterozygosity; and (4) Wilcoxon's signed rank test for excess heterozygosity (Cornuet and Luikart 1996). The latter three tests were implemented in BOTTLENECK (Piry et al. 1999). Both the sign test and Wilcoxon's signed rank test reject the null hypothesis of mutation–drift equilibrium on the basis of excess heterozygosity. However, the sign test is more conservative and simply rejects the null hypothesis based on excess heterozygosity at a significant number of loci, whereas Wilcoxon's signed rank test is more sensitive and compares the magnitude of observed heterozygosity excess with expected levels (Cornuet and Luikart 1996). Temporal replicates at each sampling site were not significantly different; we therefore combined samples from multiple years within populations to minimize the effect of small sample size on the estimated number of alleles.

The *M*-test indicates genetic bottlenecks according to the proportion of missing alleles in an allele frequency distribution. This test has the power to detect historical population bottlenecks but can be influenced by the

mutation model of the loci. Mutations of more than one step may introduce gaps in the allele distribution even if the population is large; this results in a reduced *M*-value for a population that has not experienced a bottleneck. However, a significant decrease in *M* is expected only when population bottlenecks have occurred. A population is considered to have undergone a bottleneck if its *M*-value falls below a threshold of 0.67 (Garza and Williamson 2001). Four loci (*AinA117*, *AinA2*, *AinA218*, and *AinA120*) were omitted from analyses because they did not appear to conform to a stepwise or two-phase mutation process, as evidenced by large gaps between groups of alleles for at least one of the populations. Although large gaps between groups of alleles may indicate population bottlenecks and a significant loss of alleles rather than a failure to conform to a stepwise or two-phase mutation process, elimination of these loci makes the *M*-test more conservative and reduces type I error.

An allele frequency distribution can be determined by calculating allele frequencies and counting the number of alleles within each frequency-class (e.g., 0–10%). A mode-shifted allele frequency distribution is indicated when fewer alleles are observed in the lowest frequency-class than in one or more of the intermediate frequency-classes (Luikart et al. 1998). The test for mode-shifted distributions has the greatest power to detect extremely small bottlenecks and populations that have remained small over time. Both the sign test and Wilcoxon's signed rank test use the coalescent process to simulate the distribution of heterozygosity at mutation–drift equilibrium given the specified sample and number of alleles. Heterozygosity exceeds mutation–drift equilibrium expectations when the loss of allele numbers is more rapid than the loss of heterozygosity. Data were analyzed using the two-phase model with two sets of parameters: (1) 95% single-step mutations and a variance of 12 among multiple steps, as is recommended for microsatellite data (Piry et al. 1999); and (2) 80% single-step mutations and a variance of 50 among multiple steps, which is consistent with simulations for the evolution of microsatellites in sunfishes (Neff et al. 1999). The default of 1,000 replicates was used for these analyses.

*Allelic richness*.—Petit et al. (1998) suggested that populations of higher priority for conservation efforts can be determined by considering allelic richness. To calculate allelic richness and the richness of private alleles, we used rarefaction (Kalinowski 2004) in HP-RARE (Kalinowski 2005) with a sample size of 56 genes (28 diploid individuals), which corresponded to the fewest individuals sampled from a single population and the number of genes that could be divided equally among populations to calculate overall allelic richness in the presence of balanced sample sizes. Rarefaction compensates for the increase in likelihood of sampling rare alleles as sample size increases. Populations were ranked for average allelic richness and private allelic richness across loci, and Wilcoxon's signed rank test was used to evaluate significance.

*Effective population size*.—To calculate $N_e$, we used the linkage disequilibrium method of Hill (1981) as modified by Waples (1991) and corrected to account for $N_e$ underestimation due to sampling error (England et al. 2006; Waples 2006). Effective population sizes and parametric 95% confidence intervals (CIs) were calculated in LDNe (R. S. Waples and C. Do, unpublished program); alleles at frequencies of 0.02 or less were excluded to correct for sampling error (Waples 2006).

## Results

### *General Statistics*

The number of alleles per locus ranged from 6 to 24, and the overall number of alleles per population ranged from 34 (Curved Pond) to 95 (Abbotts Lagoon) out of 153 total alleles (Table 2). Of the 153 alleles, 39 were unique to a single population; Sindicich Lagoon was the only population that did not contain unique alleles. Observed heterozygosity ranged from 0.40 to 0.74, whereas expected heterozygosity ranged from 0.41 to 0.75 (Table 2). Observed heterozygosity was lower than expected for Abbotts Lagoon ($P < 0.05$), Pyramid Lake ($P < 0.05$), and Jewel Lake ($P < 0.005$) populations. Sindicich Lagoon, Crowley Lake, and Curved Pond populations had lower observed heterozygosity than did the other five populations (all $P < 0.01$). Additionally, the observed heterozygosity of the Curved Pond population was lower than that of the Stillwater NWR population ($P < 0.05$), which in turn was lower than that of the Clear Lake Reservoir population ($P < 0.01$). The null hypothesis of Hardy–Weinberg equilibrium was rejected for two loci (*AinA120* and *AinA117*) in the Curved Pond population after sequential Bonferroni correction; therefore, all of the loci were used in further analyses. Overall linkage disequilibrium was found between two pairs of loci after Bonferroni correction. Linkage disequilibrium was significant for *AinA2* and *AinA108* in the Crowley Lake population only and for *AinA6* and *AinA212* in the Stillwater NWR, Abbotts Lagoon, Crowley Lake, Jewel Lake, and Curved Pond populations.

### *Relationships between Populations*

Using the θ estimator of $F_{ST}$, we observed significant differentiation between all pairs of populations ($P < 0.05$). No significant differentiation was observed between years within a single population; the exception

TABLE 2.—Allele frequencies and heterozygosity ($H$) measures ($H_O$ = observed; $H_E$ = expected) for 12 microsatellite loci in eight extant populations of Sacramento perch in California (SINL = Sindicich Lagoon, CLLR = Clear Lake Reservoir, ABBL = Abbotts Lagoon, CROL = Crowley Lake, JEWL = Jewel Lake, CURP = Curved Pond) and Nevada (SNWR = Stillwater National Wildlife Refuge, PYRL = Pyramid Lake). Allele sizes are given in bases.

| Locus | Allele and $H$ | SINL | SNWR | CLLR | ABBL | PYRL | CROL | JEWL | CURP |
|---|---|---|---|---|---|---|---|---|---|
| *AinA117* | 148 | | | 0.017 | 0.058 | | | | |
| | 152 | 0.015 | | 0.172 | 0.018 | | | | |
| | 156 | 0.167 | 0.086 | 0.017 | 0.079 | 0.218 | 0.191 | | 0.110 |
| | 160 | | | 0.069 | 0.011 | | | 0.083 | 0.012 |
| | 164 | | 0.114 | | 0.004 | 0.308 | | | |
| | 168 | 0.046 | | 0.017 | 0.453 | 0.013 | | | |
| | 172 | 0.546 | | 0.103 | 0.198 | 0.077 | 0.566 | 0.153 | 0.549 |
| | 176 | | | | | | | 0.181 | 0.061 |
| | 180 | | 0.300 | | | 0.167 | | | 0.024 |
| | 184 | | | | | 0.064 | | | |
| | 192 | 0.121 | 0.014 | | | 0.103 | 0.169 | | 0.098 |
| | 196 | | 0.443 | 0.190 | 0.162 | | | | |
| | 200 | | 0.029 | 0.017 | 0.014 | 0.051 | | | |
| | 204 | | | | 0.004 | | | 0.111 | |
| | 208 | | | 0.345 | | | | 0.306 | |
| | 212 | | | 0.052 | | | | 0.167 | |
| | 274 | 0.106 | 0.014 | | | | 0.074 | | 0.146 |
| | $H_O$ | 0.70 | 0.57 | 0.76 | 0.69 | 0.74 | 0.54 | 0.78 | 0.59 |
| | $H_E$ | 0.66 | 0.70 | 0.81 | 0.72 | 0.82 | 0.61 | 0.81 | 0.66 |
| *AinA2* | 190 | 0.091 | 0.257 | 0.121 | 0.305 | 0.308 | 0.293 | 0.158 | 0.171 |
| | 194 | | 0.214 | 0.190 | 0.265 | 0.397 | 0.021 | | |
| | 198 | | | 0.103 | 0.232 | | | | |
| | 202 | | | 0.207 | 0.011 | | | 0.697 | |
| | 206 | | | | 0.114 | | | | |
| | 222 | | 0.186 | 0.155 | 0.033 | 0.103 | | | |
| | 226 | 0.258 | | | | | 0.293 | | 0.585 |
| | 234 | | | | 0.037 | | | | |
| | 240 | | | | 0.004 | | | | |
| | 248 | 0.030 | 0.343 | | | 0.064 | | 0.092 | |
| | 252 | 0.621 | | | | 0.128 | 0.379 | | 0.244 |
| | 256 | | | | | | 0.014 | | |
| | 276 | | | 0.190 | | | | 0.053 | |
| | 280 | | | 0.017 | | | | | |
| | 284 | | | 0.017 | | | | | |
| | $H_O$ | 0.52 | 0.89 | 1.00 | 0.73 | 0.62 | 0.71 | 0.47 | 0.73 |
| | $H_E$ | 0.55 | 0.75 | 0.85 | 0.77 | 0.73 | 0.69 | 0.48 | 0.58 |
| *AinA203* | 278 | | | | 0.014 | | | | |
| | 282 | | | 0.069 | 0.022 | | | | |
| | 286 | | | | | | | 0.194 | |
| | 290 | 0.121 | 0.371 | 0.431 | 0.306 | 0.136 | 0.201 | 0.278 | 0.256 |
| | 294 | | | 0.103 | 0.345 | 0.015 | | 0.153 | |
| | 298 | 0.121 | | 0.086 | 0.086 | | 0.132 | | 0.220 |
| | 302 | | 0.243 | 0.103 | 0.076 | 0.409 | | | |
| | 306 | 0.758 | | 0.017 | 0.101 | | 0.667 | | 0.524 |
| | 314 | | 0.343 | 0.103 | 0.007 | 0.227 | | 0.292 | |
| | 318 | | | 0.086 | 0.043 | 0.197 | | 0.083 | |
| | 322 | | 0.043 | | | 0.015 | | | |
| | $H_O$ | 0.39 | 0.57 | 0.72 | 0.75 | 0.58 | 0.50 | 0.75 | 0.59 |
| | $H_E$ | 0.40 | 0.69 | 0.78 | 0.76 | 0.73 | 0.50 | 0.78 | 0.62 |
| *AinD119* | 179 | | 0.614 | 0.069 | 0.184 | 0.359 | | 0.105 | |
| | 187 | 0.030 | 0.314 | 0.121 | 0.025 | 0.090 | | | |
| | 191 | | 0.029 | 0.035 | 0.047 | 0.064 | | | |
| | 199 | 0.970 | 0.043 | 0.293 | 0.482 | 0.487 | 1.000 | 0.526 | 1.000 |
| | 203 | | | 0.483 | 0.263 | | | 0.224 | |
| | 207 | | | | | | | 0.105 | |
| | 211 | | | | | | | 0.040 | |
| | $H_O$ | 0.00 | 0.46 | 0.59 | 0.67 | 0.56 | 0.00 | 0.53 | 0.00 |
| | $H_E$ | 0.06 | 0.53 | 0.67 | 0.66 | 0.63 | 0.00 | 0.66 | 0.00 |
| *AinA218* | 265 | | | 0.052 | 0.007 | | | | |
| | 273 | 0.985 | 1.000 | 0.328 | 0.899 | 0.962 | 1.000 | 0.276 | 1.000 |
| | 277 | 0.015 | | 0.121 | 0.004 | 0.039 | | 0.118 | |
| | 281 | | | 0.207 | | | | 0.250 | |
| | 285 | | | 0.155 | 0.083 | | | 0.303 | |
| | 293 | | | 0.103 | 0.007 | | | 0.053 | |
| | 317 | | | 0.035 | | | | | |
| | $H_O$ | 0.03 | 0.00 | 0.79 | 0.19 | 0.08 | 0.00 | 0.68 | 0.00 |
| | $H_E$ | 0.03 | 0.00 | 0.81 | 0.18 | 0.07 | 0.00 | 0.76 | 0.00 |

TABLE 2.—Continued.

| Locus | Allele and $H$ | SINL | SNWR | CLLR | ABBL | PYRL | CROL | JEWL | CURP |
|---|---|---|---|---|---|---|---|---|---|
| *AinD106* | 222 | 0.258 | | | | | 0.444 | | 0.500 |
| | 226 | | 0.100 | | 0.210 | 0.013 | | 0.027 | |
| | 230 | | | 0.086 | 0.116 | | | 0.216 | |
| | 234 | 0.015 | 0.157 | 0.086 | 0.225 | 0.013 | | | |
| | 238 | 0.727 | 0.743 | 0.724 | 0.442 | 0.590 | 0.556 | 0.608 | 0.500 |
| | 242 | | | 0.103 | 0.007 | 0.385 | | 0.149 | |
| | $H_O$ | 0.42 | 0.40 | 0.38 | 0.71 | 0.44 | 0.36 | 0.57 | 0.45 |
| | $H_E$ | 0.41 | 0.42 | 0.46 | 0.70 | 0.51 | 0.50 | 0.57 | 0.51 |
| *AinA120* | 275 | 0.030 | 0.014 | | 0.007 | | | | |
| | 279 | 0.561 | 0.500 | 0.517 | 0.225 | 0.449 | 0.507 | 0.361 | 0.573 |
| | 283 | 0.409 | 0.457 | 0.121 | 0.011 | 0.154 | 0.493 | | 0.427 |
| | 287 | | | | | 0.039 | | | |
| | 291 | | | 0.052 | | | | | |
| | 295 | | | 0.017 | | | | | |
| | 311 | | | | 0.051 | 0.090 | | | |
| | 315 | | | 0.069 | | 0.141 | | | |
| | 319 | | | | 0.149 | | | | |
| | 323 | | 0.014 | | 0.007 | | | | |
| | 327 | | | 0.086 | | | | | |
| | 331 | | | 0.103 | 0.199 | | | | |
| | 335 | | | | 0.007 | | | | |
| | 347 | | | 0.035 | 0.312 | 0.039 | | 0.181 | |
| | 351 | | | | 0.004 | | | 0.042 | |
| | 363 | | | | | | | 0.069 | |
| | 401 | | | | | 0.064 | | | |
| | 405 | | 0.014 | | 0.007 | 0.026 | | | |
| | 409 | | | | 0.018 | | | | |
| | 413 | | | | 0.004 | | | | |
| | 417 | | | | | | | 0.111 | |
| | 445 | | | | | | | 0.069 | |
| | 449 | | | | | | | 0.139 | |
| | 453 | | | | | | | 0.028 | |
| | $H_O$ | 0.52 | 0.29 | 0.62 | 0.76 | 0.64 | 0.32 | 0.81 | 0.22 |
| | $H_E$ | 0.53 | 0.55 | 0.70 | 0.79 | 0.75 | 0.50 | 0.80 | 0.50 |
| *AinA216* | 147 | | 0.014 | | | | | | |
| | 151 | | | 0.035 | | | | | |
| | 155 | | 0.071 | 0.190 | 0.192 | 0.064 | 0.139 | 0.053 | |
| | 159 | | 0.471 | 0.035 | 0.330 | 0.128 | | 0.145 | 0.012 |
| | 163 | 0.576 | | 0.552 | 0.145 | 0.346 | 0.465 | 0.145 | 0.439 |
| | 167 | 0.121 | 0.014 | | 0.047 | | 0.201 | 0.013 | 0.317 |
| | 171 | 0.303 | 0.400 | 0.138 | 0.058 | 0.410 | 0.194 | 0.263 | 0.232 |
| | 175 | | | 0.017 | | | | 0.066 | |
| | 179 | | | 0.035 | | | | 0.079 | |
| | 187 | | | | 0.022 | | | | |
| | 191 | | 0.029 | | 0.207 | 0.051 | | 0.237 | |
| | $H_O$ | 0.45 | 0.51 | 0.66 | 0.79 | 0.77 | 0.69 | 0.82 | 0.51 |
| | $H_E$ | 0.57 | 0.62 | 0.65 | 0.79 | 0.70 | 0.69 | 0.83 | 0.66 |
| *AinA108* | 181 | | | | 0.015 | | | | |
| | 193 | | | | 0.015 | | | | |
| | 201 | | 0.271 | | | 0.103 | | | |
| | 209 | 0.015 | 0.357 | | 0.055 | 0.090 | | 0.042 | |
| | 213 | | 0.114 | 0.035 | 0.026 | 0.180 | | 0.042 | |
| | 217 | | | 0.172 | 0.386 | | | 0.375 | |
| | 221 | | | 0.207 | 0.026 | | | | |
| | 225 | | | | 0.004 | | | 0.194 | |
| | 229 | | | 0.035 | 0.147 | 0.115 | | 0.167 | |
| | 233 | 0.227 | | 0.086 | | | 0.271 | | 0.073 |
| | 237 | | 0.257 | 0.052 | 0.320 | 0.474 | | 0.014 | |
| | 241 | 0.015 | | | 0.007 | 0.026 | | | |
| | 245 | | | 0.052 | | | | | |
| | 249 | | | 0.052 | | 0.013 | | 0.167 | |
| | 253 | | | | | | | | 0.012 |
| | 257 | | | 0.241 | | | 0.014 | | |
| | 261 | 0.485 | | 0.069 | | | 0.313 | | 0.427 |
| | 265 | 0.258 | | | | | 0.396 | | 0.488 |
| | 269 | | | | | | 0.007 | | |
| | $H_O$ | 0.64 | 0.69 | 0.93 | 0.71 | 0.74 | 0.78 | 0.67 | 0.56 |
| | $H_E$ | 0.66 | 0.73 | 0.86 | 0.72 | 0.72 | 0.68 | 0.77 | 0.58 |

TABLE 2.—Continued.

| Locus | Allele and $H$ | SINL | SNWR | CLLR | ABBL | PYRL | CROL | JEWL | CURP |
|---|---|---|---|---|---|---|---|---|---|
| *AinA6* | 157 | | | | 0.036 | | | | |
| | 165 | | | | 0.004 | | | | |
| | 169 | 0.078 | | | | | 0.049 | | 0.122 |
| | 173 | | 0.029 | | 0.112 | 0.230 | | 0.290 | |
| | 177 | | | 0.121 | | | | 0.066 | |
| | 181 | | | 0.328 | 0.004 | | | 0.040 | |
| | 185 | | | 0.035 | 0.259 | 0.068 | | 0.013 | |
| | 189 | | 0.200 | 0.224 | | 0.257 | | | |
| | 193 | | 0.014 | 0.017 | | 0.095 | | | |
| | 197 | | 0.700 | 0.121 | 0.011 | 0.135 | | | |
| | 201 | 0.922 | 0.014 | 0.103 | 0.576 | 0.135 | 0.951 | 0.079 | 0.878 |
| | 205 | | 0.043 | 0.052 | | 0.041 | | 0.342 | |
| | 213 | | | | | | | 0.171 | |
| | 217 | | | | | 0.041 | | | |
| | $H_O$ | 0.16 | 0.51 | 0.79 | 0.55 | 0.84 | 0.10 | 0.68 | 0.24 |
| | $H_E$ | 0.15 | 0.47 | 0.81 | 0.59 | 0.84 | 0.09 | 0.77 | 0.22 |
| *AinA212* | 298 | 0.677 | | | | | 0.679 | | 0.390 |
| | 310 | | | 0.224 | | 0.014 | | 0.092 | |
| | 318 | | 0.029 | 0.103 | 0.027 | 0.186 | | 0.040 | |
| | 322 | | 0.014 | | 0.027 | 0.200 | | 0.013 | |
| | 326 | 0.177 | 0.343 | | 0.015 | 0.229 | 0.279 | 0.053 | 0.500 |
| | 330 | | | 0.293 | 0.648 | 0.029 | | 0.079 | |
| | 334 | | 0.029 | 0.103 | 0.159 | 0.029 | | 0.316 | |
| | 338 | 0.145 | 0.143 | 0.103 | 0.004 | 0.071 | 0.043 | 0.316 | 0.110 |
| | 342 | | | 0.017 | 0.121 | | | 0.092 | |
| | 346 | | 0.443 | 0.155 | | 0.243 | | | |
| | $H_O$ | 0.42 | 0.77 | 0.86 | 0.52 | 0.83 | 0.57 | 0.76 | 0.56 |
| | $H_E$ | 0.50 | 0.67 | 0.82 | 0.54 | 0.82 | 0.46 | 0.78 | 0.59 |
| *AinD101* | 119 | | | | 0.029 | | | | |
| | 123 | | 0.357 | 0.035 | 0.270 | 0.128 | | | |
| | 127 | 0.016 | 0.414 | | 0.022 | 0.128 | 0.007 | | |
| | 131 | | 0.071 | 0.035 | 0.037 | 0.218 | | 0.122 | |
| | 135 | | 0.157 | 0.414 | 0.438 | 0.077 | | 0.338 | |
| | 139 | 0.339 | | 0.103 | 0.011 | 0.013 | 0.707 | | 0.573 |
| | 143 | | | 0.103 | 0.080 | | | 0.405 | |
| | 147 | 0.645 | | | | | 0.279 | | 0.427 |
| | 151 | | | 0.276 | 0.110 | 0.115 | | 0.122 | |
| | 155 | | | 0.035 | 0.004 | 0.026 | | 0.014 | |
| | 163 | | | | | 0.295 | 0.007 | | |
| | $H_O$ | 0.52 | 0.69 | 0.72 | 0.68 | 0.72 | 0.37 | 0.54 | 0.61 |
| | $H_E$ | 0.48 | 0.68 | 0.74 | 0.72 | 0.82 | 0.43 | 0.70 | 0.50 |
| | Overall number of alleles | 39 | 54 | 89 | 95 | 73 | 36 | 72 | 34 |
| | Overall $H_O$ | 0.40 | 0.53 | 0.74 | 0.65 | 0.63 | 0.41 | 0.67 | 0.42 |
| | Overall $H_E$ | 0.41 | 0.57 | 0.75 | 0.66 | 0.68 | 0.43 | 0.73 | 0.45 |

TABLE 3.—Pairwise genetic differentiation index ($F_{ST}$; as estimated by the θ of Weir and Cockerham 1984) values calculated for eight extant populations of Sacramento perch in California and Nevada (population codes defined in Table 2). Bootstrapped 95% confidence intervals are shown in parentheses.

| Population | SINL | SNWR | CLLR | ABBL | PYRL | CROL | JEWL |
|---|---|---|---|---|---|---|---|
| SNWR | 0.39 (0.28–0.49) | | | | | | |
| CLLR | 0.29 (0.21–0.36) | 0.21 (0.15–0.26) | | | | | |
| ABBL | 0.27 (0.22–0.33) | 0.22 (0.16–0.29) | 0.14 (0.10–0.19) | | | | |
| PYRL | 0.28 (0.20–0.34) | 0.13 (0.10–0.16) | 0.14 (0.10–0.19) | 0.16 (0.12–0.20) | | | |
| CROL | 0.05 (0.01–0.10) | 0.39 (0.27–0.53) | 0.32 (0.22–0.42) | 0.28 (0.22–0.34) | 0.29 (0.21–0.37) | | |
| JEWL | 0.32 (0.25–0.39) | 0.25 (0.19–0.31) | 0.10 (0.06–0.13) | 0.17 (0.11–0.24) | 0.17 (0.12–0.24) | 0.34 (0.27–0.42) | |
| CURP | 0.07 (0.03–0.11) | 0.37 (026–0.48) | 0.29 (0.22–0.36) | 0.26 (0.21–0.31) | 0.27 (0.21–0.32) | 0.03 (0.01–0.06) | 0.31 (0.25–0.38) |

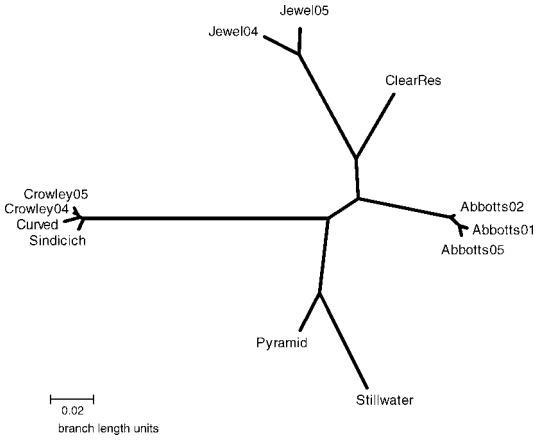


FIGURE 2.—Neighbor-joining dendrogram of genetic relationships based on Cavalli-Sforza and Edwards' (1967) chord distances ($D_{CSE}$) calculated for eight extant Sacramento perch populations (Abbotts Lagoon, Jewel Lake, Sindicich Lagoon, Curved Pond, Clear Lake Reservoir [ClearRes], and Crowley Lake in California; Pyramid Lake and Stillwater National Wildlife Refuge in Nevada). All $D_{CSE}$ values were significant ($P < 0.05$) except those between annual samples from the Abbotts Lagoon (2001, 2002, and 2005) or Crowley Lake (2004 and 2005) population.

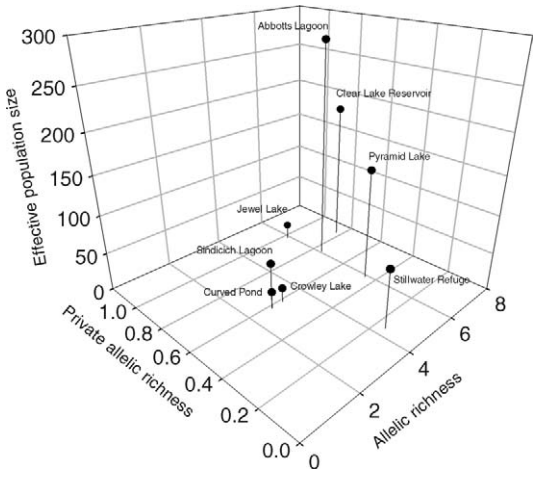


FIGURE 3.—Three-dimensional plot of effective population size, allelic richness, and richness of private alleles in eight extant populations of Sacramento perch (Figure 1), illustrating the relative utility of each stock as a source for reintroduction. Populations with greater potential value as sources are found in the upper back quadrant.

was for Jewel Lake, where the 2004 sample consisted of only 14 individuals. Values of θ ranged from nonsignificant (between years from the same location) to 0.39 (Table 3). Values of $D_{CSE}$ and θ produced similar relationships between populations (Figure 2; Table 3). Global θ for all populations was 0.24 (95% CI = 0.20–0.28), indicating that 24% of the genetic variation in the species resulted from variation among populations.

### *Tests for Population Bottlenecks*

Significant population bottlenecks were detected via the *M*-test in five populations: Sindicich Lagoon, Stillwater NWR, Crowley Lake, Pyramid Lake, and Curved Pond (Table 4). When all 12 loci were included in the analysis, the Jewel Lake population also fell below the criterion for determining a bottleneck (i.e., $M < 0.67$). Eliminating loci that failed to conform to Hardy–Weinberg equilibrium or that were in linkage disequilibrium did not significantly affect *M*-values. Using the model with 95% stepwise mutations and a variance of 12, bottlenecks were indicated in the populations at Jewel Lake (Wilcoxon's signed rank test for excess heterozygosity) and Curved Pond (test for mode-shifted distribution). When samples were analyzed based on individual years, a bottleneck was also indicated in the 2005 sample from Crowley Lake (sign test and Wilcoxon's signed rank test). Using the model

TABLE 4.—Genetic diversity as measured by observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), *M*-statistic (represents loss of alleles; bold values indicate population bottlenecks), allelic richness (rank in parentheses; sample size = 56 genes), private allelic richness (rank in parentheses), and effective population size ($N_e$; parametric 95% confidence interval [CI] in parentheses) of eight extant Sacramento perch populations in California and Nevada (Table 1).

| Population | $H_O$ | $H_E$ | *M* | Allelic richness (rank) | Private allelic richness (rank) | $N_e$ (95% CI) |
|---|---|---|---|---|---|---|
| Sindicich Lagoon | 0.40 | 0.41 | **0.50** | 3.17 (6) | 0.01 (8) | 20 (11–40) |
| Stillwater National Wildlife Refuge | 0.53 | 0.57 | **0.65** | 4.31 (5) | 0.13 (5) | 78 (37–633) |
| Clear Lake Reservoir | 0.74 | 0.75 | 0.83 | 7.38 (1) | 0.79 (2) | 184 (75–infinite) |
| Abbotts Lagoon | 0.65 | 0.66 | 0.80 | 6.07 (2) | 0.75 (3) | 281 (184–539) |
| Pyramid Lake | 0.63 | 0.68 | **0.67** | 5.89 (2) | 0.4 (4) | 146 (72–9,618) |
| Crowley Lake | 0.41 | 0.43 | **0.52** | 2.76 (6) | 0.09 (6) | 22 (14–36) |
| Jewel Lake | 0.67 | 0.73 | 0.70 | 5.88 (2) | 0.95 (1) | 21 (17–27) |
| Curved Pond | 0.42 | 0.45 | **0.58** | 2.75 (6) | 0.06 (7) | 61 (24–infinite) |
| Total | | | | 9.21 | | |

with 80% stepwise mutations and a variance of 50, bottlenecks were indicated in the populations at Jewel Lake (sign test), Curved Pond (Wilcoxon's signed rank test), and Pyramid and Crowley lakes (Wilcoxon's signed rank test). None of the tests indicated genetic bottlenecks in the Clear Lake Reservoir or Abbotts Lagoon population.

*Allelic Richness*

The greatest allelic richness was found in the Clear Lake Reservoir population, followed by the Abbotts Lagoon, Pyramid Lake, and Jewel Lake populations ($P > 0.05$ for differences in richness; Table 4). The greatest private allelic richness was found in the Jewel Lake, Clear Lake Reservoir, and Abbotts Lagoon populations; however, private allelic richness did not differ significantly between populations (Table 4).

*Effective Population Size*

Three populations—Clear Lake Reservoir, Pyramid Lake, and Abbotts Lagoon—had $N_e$ values that exceeded 50 and 95% CIs that did not overlap 50. Two additional populations, Stillwater NWR and Curved Pond, had estimated $N_e$ values greater than 50 and 95% CIs that included 50. The remaining three populations had $N_e$ values between 19 and 23 (Table 4).

## Discussion

Significant differences were observed in genetic diversity within and among Sacramento perch populations and in the $N_e$ of the populations. Only two of the eight populations (Abbotts Lagoon and Clear Lake Reservoir) exhibited no evidence of genetic bottlenecks. Only three populations (Abbotts Lagoon, Clear Lake Reservoir, and Pyramid Lake) had an $N_e$ greater than the minimum recommended to prevent 1% inbreeding. The eight study populations had wide ranges of heterozygosity and allelic richness. The differences among extant populations may have resulted from variation in the genetic diversity of their founding populations or from variation in the number of founding individuals. Heterozygosity levels were similar to those found in populations of other centrarchids (e.g., Coughlin et al. 2003; Stepien et al. 2007). A positive correlation between fitness and heterozygosity has been observed (e.g., Rowe et al. 1999); therefore, fitness is potentially greater in the Sacramento perch populations with greater heterozygosity (i.e., Clear Lake Reservoir, Abbotts Lagoon, Pyramid Lake, and Jewel Lake). Populations were strongly differentiated, suggesting either differentiation among the source populations used for stocking or a strong signature of genetic drift resulting from genetic bottlenecks.

*Population Bottlenecks*

Six of the eight populations evaluated for use in restoration showed evidence of a genetic bottleneck in at least one test. Differences in results among the four tests for bottlenecks may have resulted from the power of each test to detect bottlenecks that occurred in the past or that occurred over multiple generations. Simulations by Williamson-Natesan (2005) indicated that methods evaluating excess heterozygosity (e.g., the sign test and Wilcoxon's signed rank test) have the highest likelihood of detecting a population bottleneck if it was recent and of low severity and if the prebottleneck value of $\theta$ ($4N_e\mu$) is small. In contrast, the *M*-test is more likely to detect a bottleneck if it has lasted for multiple generations, if the population has recovered, or if the prebottleneck $\theta$ is large. These detection differences may explain why genetic bottlenecks were indicated by three of four tests for the Curved Pond and Crowley Lake populations, two tests for the Jewel and Pyramid Lake populations, and one test for the Sindicich Lagoon and Stillwater NWR populations. It is possible that a bottleneck would erroneously be indicated by the *M*-test if multiple populations containing alleles at opposite ends of the allelic distribution were used to found the extant populations; however, we have no reason to believe that this is the case. Abbotts Lagoon and Clear Lake Reservoir populations were the only populations in which bottlenecks were not detected. It is possible that a bottleneck would not be indicated by the four tests if a population was founded by fish from two or more sources (e.g., DeYoung et al. 2003), but again, we have no reason to believe that such is the case for these populations.

Genetic bottlenecks are common in species introductions (e.g., Ramey et al. 2000; Lambert et al. 2005) and have also been observed in other populations of conservation concern; however, many of the latter bottlenecks are the result of population declines rather than translocations. O'Grady et al. (2006) suggested that bottlenecks have fitness consequences for a population and can increase the risk of extinction by causing the expression of recessive deleterious alleles. Loss of genetic diversity, a likely consequence of a population bottleneck, is related to reduced population fitness (Reed and Frankham 2003), and the mean fitness of individuals in a population directly impacts the dynamics of that population (Reed et al. 2007a, 2007b). However, the prevalence of deleterious alleles in Sacramento perch is unknown, and populations have

survived in some of these stocked locations for over 100 years.

*Implications for Reintroductions*

The Society for Ecological Restoration recommended the use of large, genetically diverse source populations or drawing from several stocks to establish a diverse population that will have the greatest chance of succeeding in the new location (Falk et al. 2001). Logically, stocking from multiple sources is the most effective way to maximize genetic variation in introduced populations and thereby minimize the risk of inbreeding depression. However, founder effects and subsequent genetic drift, combined with ecological differentiation among extant populations and differentiation among historical populations, create the potential for outbreeding depression if multiple stocks are interbred at reintroduction sites (e.g., Fischer and Matthies 1997; Gharrett et al. 1999).

Because this is the first research to address the genetic diversity of Sacramento perch, the results presented here are the primary basis for selecting populations to use as sources for reintroduction. Additional research on local adaptation, phenotypic differentiation, and phenotypic plasticity would be beneficial in selecting sources that are best suited to the characteristics of each reintroduction site. Both genotypic and phenotypic differentiation may be the result of either historical processes or post-translocation genetic drift. Outbreeding depression is more likely to be of concern in restoration efforts if differentiation is the result of historical processes, as individuals that are adapted to one set of environmental conditions might not persist at restoration sites with different conditions. In contrast, if differentiation is the result of genetic drift, then Sacramento perch are more likely to become self sustaining as long as diversity in reintroduced populations is sufficient. Mating experiments could assist in predicting whether inbreeding or outbreeding depression is more likely and would provide guidance in the choice between a single source and multiple sources for stocking at a given reintroduction site.

Without such data, however, we are limited to selecting sources on the basis of our current results. Therefore, to visualize the potential value of each extant Sacramento perch population as a reintroduction source, we graphed populations (SigmaPlot version 9) as a function of allelic richness, private allelic richness, and $N_e$ (Figure 3). For this purpose, we reanalyzed private allelic richness for the combined Sindicich Lagoon, Crowley Lake, and Curved Pond samples, because alleles that were exclusive to this set of populations often were found in more than one of the populations and thus were not reflected in private allelic richness. Use of private allelic richness to determine the value of each population as a separate potential source would therefore fail to consider the genetic diversity that was exclusive to these samples.

Examination of Figure 3 indicates that two populations are clearly preferable as source populations: Abbotts Lagoon and Clear Lake Reservoir. These populations had the greatest allelic richness and largest $N_e$ values, as well as high private allelic richness. However, these populations do not represent the full diversity of the species, making it necessary to draw from other populations to retain diversity at reintroduction sites. For example, the Jewel Lake population has the highest private allelic richness, making it a potentially valuable source because of its uniqueness. The Pyramid Lake population had high allelic richness and a relatively large $N_e$; its private allelic richness would have been higher if the Stillwater NWR population had been excluded from analyses. Even the populations that have experienced the most severe bottlenecks (Sindicich Lagoon, Crowley Lake, and Curved Pond) may be useful as sources for reintroduction. Although stocking from the more-diverse sources would be more likely to result in self-sustaining populations (Ramey et al. 2000), the Sindicich Lagoon, Crowley Lake, and Curved Pond populations are genetically distinct from the others and each may thus provide some unique genetic contribution.

These results can form the basis for a number of reintroduction strategies. For example, individuals can be drawn from either or both of the two most diverse populations or from combinations of more- and less-diverse populations. If diversity at the reintroduction site is a priority, a combination of populations could be used as sources, preferably including both of the most diverse populations. Diversity may be considered a priority when establishing a new population because it forms the basis of the population's ability to adapt to its new habitat. Increasing genetic diversity may therefore increase the likelihood that a new population will be established successfully. Reintroducing individuals from multiple populations to a single location would also minimize the risk of inbreeding depression by allowing unrelated individuals to mate, thereby reducing the likelihood that offspring will possess two copies of a deleterious recessive allele. Although the true risk of inbreeding depression cannot be stated without extensive monitoring of extant populations, evidence of low genetic diversity and genetic bottlenecks in many of the populations suggests the potential for inbreeding depression. Inbreeding depression has been documented as leading to decreases in survival,

reproduction, population size, and time to extinction (O'Grady et al. 2006). Therefore, minimizing inbreeding depression may increase the likelihood that a reintroduced population will survive and reproduce.

Mixing of multiple populations could result in outbreeding depression, as has been documented in several species across a range of taxa (e.g., Gharrett et al. 1999; Peer and Taborsky 2005). The most likely cause of outbreeding depression in the present situation would be the breeding of stocked individuals originating from different, locally adapted ancestral populations. Although the true risk of outbreeding depression cannot be stated without experimental mating and survival data (e.g., Gharrett et al. 1999), high $F_{ST}$ values between pairs of Sacramento perch populations suggest that outbreeding depression could occur. Effects of outbreeding depression, such as decreased reproduction and survival rates, can be correlated with genetic divergence and a lack of interbreeding between populations (Edmands 1999).

If minimizing the risk of outbreeding depression is a priority, then using just one of the two most diverse populations as the source for any given reintroduction should be considered. Although the use of a single population as a source could increase the risk of inbreeding depression, generations of breeding of closely related individuals can reduce inbreeding depression (e.g., Peer and Taborsky 2004). Therefore, it is possible that deleterious alleles have been purged from Sacramento perch populations, resulting in relative robustness to inbreeding depression. Although diversity in a newly established population will be lower from use of a single source than from use of multiple sources, the single-source approach would balance the risks of inbreeding depression, outbreeding depression, and low genetic diversity when the magnitude of these risks is unknown. Additionally, the data suggest that Sacramento perch can persist even after experiencing a genetic bottleneck; therefore, a lack of diversity does not necessarily preclude the possibility of establishing a self-sustaining population.

Because any one of these reintroduction strategies can result in successful establishment or in failure, we suggest a policy of active, adaptive management to determine the most successful reintroduction strategies. Under active, adaptive management, a range of reintroduction strategies using different source populations would be tested to determine which strategies lead to successful establishment. For example, some reintroduction sites can be stocked from a single source while others receive fish from several sources.

The genetic baseline established in this study can be used to monitor newly established populations. The contribution of different sources to future generations can be measured using admixture analysis (e.g., Lutz-Carrillo et al. 2006), and the contribution of particular individuals to future generations can be determined via parentage analysis (e.g., Zhu et al. 2002). Even translocations of large numbers of individuals can result in bottlenecks if only a few stocked individuals survive and contribute to further generations (Stockwell et al. 1996). Analysis of the relative contributions of stocking sources at particular sites is useful in identifying which sources (separately or in combination) are more successful in particular locations and whether further supplementation is necessary to maintain genetic diversity. For example, when genetic markers were used to identify lake trout *Salvelinus namaycush* that were reintroduced to Lake Ontario (Perkins et al. 1995), various strains were found to have unequal contributions at different locations; subsequent stocking efforts were thus able to focus on use of the successful strains. Genetic monitoring can also identify when particular individuals have a disproportionately high contribution to subsequent generations. Unequal parental contribution is known to severely reduce genetic diversity by substantially increasing inbreeding and genetic drift (Rodriguez-Ramilo et al. 2006). The potential for outbreeding depression begins with the $F_2$ generation (Edmands 1999; Edmands and Timmerman 2003); therefore, populations should be monitored for multiple generations before an assessment of the most effective reintroduction strategies is conducted.

In conclusion, our results highlight the frequency of genetic bottlenecks in newly established populations of species that require conservation. Only two of the eight evaluated Sacramento perch populations showed no evidence of genetic bottlenecks and met the recommended minimum $N_e$ for short-term adaptation. Establishing this genetic baseline is a first step toward incorporating genetic considerations into reintroduction plans. Additionally, this baseline will be useful in monitoring different management strategies and informing future genetic supplementation of new populations.

## Acknowledgments

Samples were provided by P. Crain, C. Woodley, R. Piaskowski, S. Chandra, K. Grubb, C. Miller, M. Johnson, and P. Alexander. Invaluable comments on this manuscript were given by C. Floyd, M. Baerwald, J. Hull, P. Moyle, H. Ernest, and anonymous reviewers. Members of the Genomic Variation Laboratory in the Department of Animal Science, University of California–Davis (UC–Davis), provided laboratory and analysis assistance and helpful discussions. This research was supported by a CALFED Ecosystem

Restoration Program grant (O2-P34) from CBDA to P. Moyle, J. Cech, and B. May. Support for R.S.S. was provided by the aforementioned CBDA grant; the UC–Davis Ecology Graduate Group; and Hart, Cole, and Goss summer fellowships from the UC–Davis Department of Animal Science.

## References

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2004. GENETIX 4.05, logiciel sous Windows pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550–570.

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.

Coughlin, W. D., A. A. Echelle, R. A. Van Den Bussche, L. M. Cofer, and W. L. Fisher. 2003. Genetic structure of spotted bass (*Micropterus punctulatus*) in the Red and Arkansas River basins: microsatellite and mitochondrial DNA variation. Southwestern Naturalist 48:526–533.

DeYoung, R. W., S. Demarais, R. L. Honeycutt, A. P. Rooney, R. A. Gonzales, and K. L. Gee. 2003. Genetic consequences of white-tailed deer (*Odocoileus virginianus*) restoration in Mississippi. Molecular Ecology 12:3237–3252.

Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757–1768.

Edmands, S., and C. C. Timmerman. 2003. Modeling factors affecting the severity of outbreeding depression. Conservation Biology 17:883–892.

England, P. R., J. M. Cornuet, P. Berthier, D. A. Tallmon, and G. Luikart. 2006. Estimating effective population size from linkage disequilibrium: severe bias in small samples. Conservation Genetics 7:303–308.

Falk, D. A., E. Knapp, and E. O. Guerrant. 2001. An introduction to restoration genetics. Prepared by the Society for Ecological Restoration for U.S. Environmental Protection Agency, Plant Conservation Alliance, and Bureau of Land Management, Tucson, Arizona.

Fischer, M., and D. Matthies. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84:1685–1692.

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.

Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, and S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-brood year pink salmon. Aquaculture 173:117–129.

Gobalet, K. W., P. D. Schulz, T. A. Wake, and N. Siefkin. 2004. Archaeological perspectives on native American fisheries of California, with emphasis on steelhead and salmon. Transactions of the American Fisheries Society 133:801–833.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). University of Lausanne, Switzerland. Available: www2.unil.ch/popgen/softwares/fstat.htm. (July 2005).

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Hill, W. G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genetical Research 38:209–216.

Holsinger, K. E., and P. Vitt. 1997. The future of conservation biology: what's a geneticist to do? Pages 202–216 *in* S. T. Pickett, editor. The ecological basis of conservation: heterogeneity, ecosystems, and biodiversity. Chapman and Hall, New York.

IUBMB (International Union of Biochemistry and Molecular Biology). 1992. Enzyme nomenclature 1992. Academic Press, San Diego, California.

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 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187–189.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5:150–163.

Lambert, D. M., T. King, L. D. Shepherd, A. Livingston, S. Anderson, and J. L. Craig. 2005. Serial population bottlenecks and genetic variation: translocated populations of the New Zealand Saddleback (*Philesturnus carunculatus rufusater*). Conservation Genetics 6:1–14.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9:782–791.

Luikart, G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238–247.

Lutz-Carrillo, D. J., C. C. Nice, T. H. Bonner, M. R. J. Forstner, and L. T. Fries. 2006. Admixture analysis of Florida largemouth bass and northern largemouth bass using microsatellite loci. Transactions of the American Fisheries Society 135:779–791.

Marchetti, M. P. 1999. An experimental study of competition between the native Sacramento perch (*Archoplites interruptus*) and introduced bluegill (*Lepomis macrochirus*). Biological Invasions 1:55–65.

Maudet, C., C. Miller, B. Bassano, C. Breitenmoser-Würsten, D. Gauthier, G. Obexer-Ruff, J. Michallet, P. Taberlet, and G. Luikart. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex* (*ibex*)]. Molecular Ecology 11:421–436.

Moyle, P. B. 2002. Inland fishes of California, 2nd edition. University of California Press, Berkeley.

Moyle, P. B., R. Pine, L. R. Brown, C. H. Hanson, B. Herbold, K. M. Lentz, L. Meng, J. J. Smith, D. A.

Sweetnam, and L. Winternitz. 1996. Recovery plan for the Sacramento–San Joaquin Delta native fishes. U.S. Fish and Wildlife Service, Portland, Oregon.

Neff, B. D., P. Fu, and M. R. Gross. 1999. Microsatellite evolution in sunfish (Centrarchidae). Canadian Journal of Fisheries and Aquatic Sciences 56:1198–1205.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283–292.

O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133:42–51.

Peer, K., and M. Taborsky. 2004. Female ambrosia beetles adjust their offspring sex ratio according to outbreeding opportunities for their sons. Journal of Evolutionary Biology 17:257–264.

Peer, K., and M. Taborsky. 2005. Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. Evolution 59:317–323.

Perkins, D. L., J. D. Fitzsimons, J. E. Marsden, C. C. Krueger, and B. May. 1995. Differences in reproduction among hatchery strains of lake trout at eight spawning areas in Lake Ontario: genetic evidence from mixed-stock analysis. Journal of Great Lakes Research 21:364–374.

Petit, R. J., A. El Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12:844–855.

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. Journal of Heredity 90:502–503.

Pyron, M. 1999. Relationships between geographical range size, body size, local abundance, and habitat breadth in North American suckers and sunfishes. Journal of Biogeography 26:549–558.

Ramey, R. R., G. Luikart, and F. J. Singer. 2000. Genetic bottlenecks resulting from restoration efforts: the case of bighorn sheep in Badlands National Park. Restoration Ecology 8:85–90.

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.

Reed, D. H., A. C. Nicholas, and G. E. Stratton. 2007a. Genetic quality of individuals impacts population dynamics. Animal Conservation 10:275–283.

Reed, D. H., A. C. Nicholas, and G. E. Stratton. 2007b. Inbreeding levels and prey abundance interact to determine fecundity in natural populations of two species of wolf spider. Conservation Genetics 8:1061–1071.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Robichaux, R. H., E. A. Friar, and D. W. Mount. 1997. Molecular genetic consequences of a population bottleneck associated with reintroduction of the Mauna Kea silversword (*Argyroxiphium sandwicense* ssp. sandwicense [Asteraceae]). Conservation Biology 11:1140–1146.

Rodriguez-Ramilo, S. T., P. Moran, and A. Caballero. 2006. Relaxation of selection with equalization of parental contributions in conservation programs: an experimental test with *Drosophila melanogaster*. Genetics 172:1043–1054.

Rowe, G., T. J. C. Beebee, and T. Burke. 1999. Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. Animal Conservation 2:85–92.

Schwartz, R. S., and B. May. 2004. Characterization of microsatellite loci in Sacramento perch (*Archoplites interruptus*). Molecular Ecology Notes 4:694–697.

Skinner, J. 1962. A historical review of the fish and wildlife resources of the San Francisco Bay area. California Department of Fish and Game, Water Projects Branch, Report 1, Sacramento.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Stepien, C. A., D. J. Murphy, and R. M. Strange. 2007. Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: an interplay of behaviour and geography. Molecular Ecology 16:1605–1624.

Stockwell, C. A., M. Mulvey, and G. L. Vinyard. 1996. Translocations and the preservation of allelic diversity. Conservation Biology 10:1133–1141.

Waples, R. S. 1991. Genetic methods for estimating the effective size of cetacean populations. International Whaling Commission Report of the Commission 13:279–300.

Waples, R. S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics 7:167–184.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Williamson-Natesan, E. G. 2005. Comparison of methods for detecting bottlenecks from microsatellite loci. Conservation Genetics 6:551–562.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19:395–420.

Zhu, B., F. Zhou, H. Cao, Z. Shao, B. May, and J. Chang. 2002. Analysis of genetic variation in the Chinese sturgeon, *Acipenser sinensis*: estimating the contribution of artificially produced larvae in a wild population. Journal of Applied Ichthyology 18:301–306.