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Assessment of Genetic Diversity and Hybridization for the Endangered Conasauga Logperch (*Percina jenkinsi*)

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Assessment of Genetic Diversity and Hybridization for the Endangered Conasauga Logperch (*Percina jenkinsi*)

Abstract

The Conasauga logperch, *Percina jenkinsi* is one of the rarest darters in North America afforded protection under the Endangered Species Act. Unfortunately, little is known about potential threats to the genetic diversity of this species, a narrow endemic. Loss of genetic diversity, spawning of closely related individuals, and hybridization with closely related congeners have been known to increase the rate of extinction for threatened or endangered taxa. We evaluated these risks by estimating and comparing levels of genetic diversity between *P. jenkinsi* and *P. kathae* (a closely related, morphologically similar, and more abundant congener) using twelve microsatellite loci. Specifically, we assessed whether a recent genetic bottleneck occurred in *P. jenkinsi*, determined the potential threat of hybridization between *P. jenkinsi* and *P. kathae*, and evaluated the maintenance of genetic diversity among *P. jenkinsi* collected in the wild, as broodstock for an experimental hatchery program, and their progeny. Estimates of genetic diversity between *P. jenkinsi* and *P. kathae* showed no significant differences in average number of alleles (7.083 vs. 9.5; $P = 0.26$), average observed heterozygosity (0.646 vs. 0.600, $P = 0.64$), or average expected heterozygosity (0.634 vs. 0.627, $P = 0.86$). Estimated N_e for *P. jenkinsi* and *P. kathae* was 114 (95% CI 60-526) and -497 (95% CI 264-infinity). We found no evidence of hybridization between *P. jenkinsi* and *P. kathae* and there was no detectable genetic signal of a recent genetic bottleneck in *P. jenkinsi* or *P. kathae*. Comparisons of observed and expected heterozygosity between *P. jenkinsi* collected in the wild, chosen as brood, and their progeny were similar; however, there was a 32% reduction in number of alleles (i.e., a loss of 16 of 50 alleles) due to hatchery influences. Specifically, twelve alleles (24% reduction) were lost between wild and hatchery broodstock, with the remainder being lost between hatchery brood and their respective offspring (note that the majority of alleles lost among groups were at observed frequencies < 0.05). Results of parentage analysis for hatchery *P. jenkinsi* showed that each male and female broodstock contributed offspring. The average number of offspring for the seven males and two females used as broodstock was 6.71 and 23.5. Based on the number of male and female broodstock, the predicted N_e of the offspring was 6.22 and by incorporating the mean and variance in progeny number, the observed N_e size was 4.97. The relatively high levels of genetic diversity coupled with the estimate of N_e indicated that the hatchery program was successful at minimizing the reduction in N_e between brood and progeny; however, the observed 32% loss of alleles between the wild *P. jenkinsi* and progeny of hatchery broodstock is alarming. This loss was due to sampling too few broodstock. Fortunately this loss can be mitigated should this program continue in the future by using larger broodstock collections over multiple years.

Keywords

reintroduction; captive breeding; conservation aquaculture; Percidae; darter

Cover Page Footnote

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INTRODUCTION

The Conasauga Logperch, *Percina jenkinsi*, is one of the rarest darters in North America and found only from a 44-km reach of the Conasauga River (Figure 1), a tributary of the Coosa River in the Mobile Basin, near the Georgia/Tennessee state line (Etnier and Starnes 1993). It is unusually restricted when compared to other Coosa River endemics (Thompson 1985; George et al. 2010), and because of its restricted distribution and low abundance, *P. jenkinsi* was listed as Federally Endangered in 1985 (USFWS 1985).

While there are no historical records indicating that *P. jenkinsi* ever occupied a more extensive range, the occurrence of sympatric taxa with more widespread distributions suggests that their rarity may be relatively recent and potentially caused by competition with a sympatric member of the subgenus, *P. kathae* (widespread throughout the Mobile Basin; Thompson 1985). Competition with a sympatric congener can have varying outcomes (Moyer et al. 2005), but one potential outcome is the homogenization of two separate taxa via hybridization (Epifanio and Philipp 2001; Scribner et al. 2001; Hasselman et al. 2014). While the threat of hybridization is often from an invasive or translocated taxon (Rhymer and Simberloff 1996; Allendorf et al. 2001; McKinney 2006), anthropogenic events such as habitat alteration have been shown to increase the rate of hybridization among sympatric species (Broughton et al. 2011; Crego-Prieto et al. 2012). Such hybridization between *P. jenkinsi* and *P. kathae* potentially further endangers *P. jenkinsi*. Therefore, there is a need to document and evaluate the threat of hybridization for *P. jenkinsi*.

The restricted distribution and low abundance of *P. jenkinsi* (Hagler et al. 2011) has warranted the development of captive propagation protocols for this endangered organism. In 2002, the first attempt at captive propagation was unsuccessful, despite extensive experience in propagating closely-related species. However, in 2011, over 700 offspring were produced from nine *P. jenkinsi* broodstock (note that the sex of each was unknown). Although the propagation effort appears successful in terms of the number of offspring produced, perceived genetic risks (i.e., loss of genetic diversity due to inbreeding or genetic drift) should be evaluated. For example, the source population generally should have a high degree of genetic diversity and genetic similarity to that of the new or recipient population to offset the potential decrease in average fitness associated with inbreeding and/or a loss of genetic variation (Miller and Kapuscinski 2003).

Initial assessments of genetic diversity can also help to inform conservation and management actions before they are taken and define a reference point for continued monitoring efforts, which in turn give quantitative information that can aid in the implementation of adaptive management. Specifically, genetic monitoring can provide: 1) an understanding of the present and historical levels of genetic diversity in a population or species (e.g., prior to release of hatchery individuals); 2) an assessment of the alteration of these characteristics (i.e., perhaps due to anthropogenic factors); and 3) an evaluation of the biological consequences of management and conservation initiatives (Schwartz et al. 2007, Laikre et al. 2010).

The objectives of this study were to: 1) estimate and compare levels of genetic diversity between *P. jenkinsi* and *P. kathae* within the zone of sympatry; 2) determine the potential threat of hybridization between *P. jenkinsi* and *P. kathae*; 3) assess whether a recent genetic bottleneck occurred in *P. jenkinsi*; 4) establish a genetic baseline dataset (i.e., estimate average number of alleles, heterozygosity, and effective population size) for future genetic monitoring of *P. jenkinsi*; and 5) evaluate the maintenance of genetic diversity among wild, hatchery broodstock, and progeny from hatchery broodstock.

METHODS

Tissue collections were conducted by Conservation Fisheries, Inc. and Tennessee Aquarium Conservation Institute via mask and snorkel during August 2010 (for broodstock) and April-June 2012 (for remaining *P. jenkinsi* and all *P. kathae*). Collection methods were adapted from Dinkins and Shute (1996), using a small group of snorkelers to corral logperch until they could either be driven into a small handnet from a resting position on the bottom or guided into a seine by the snorkeling team. For both species, fish were collected over a 24 km stretch of river where they co-occur (Figure 1; note that due to the rarity of this species, we refrained from publishing exact locality information). Fin clips and photo vouchers were taken from all captured individuals. Photo vouchers were archived at the Tennessee Aquarium Conservation Institute. All tissue samples were placed in 95% non-denatured ethanol and archived at the United States Fish and Wildlife Service Conservation Genetics Lab in Warm Springs, GA. Genomic DNA was extracted from each fin clip using the DNeasy Blood and Tissue kit (QIAGEN, Inc., Valencia, California) protocol.

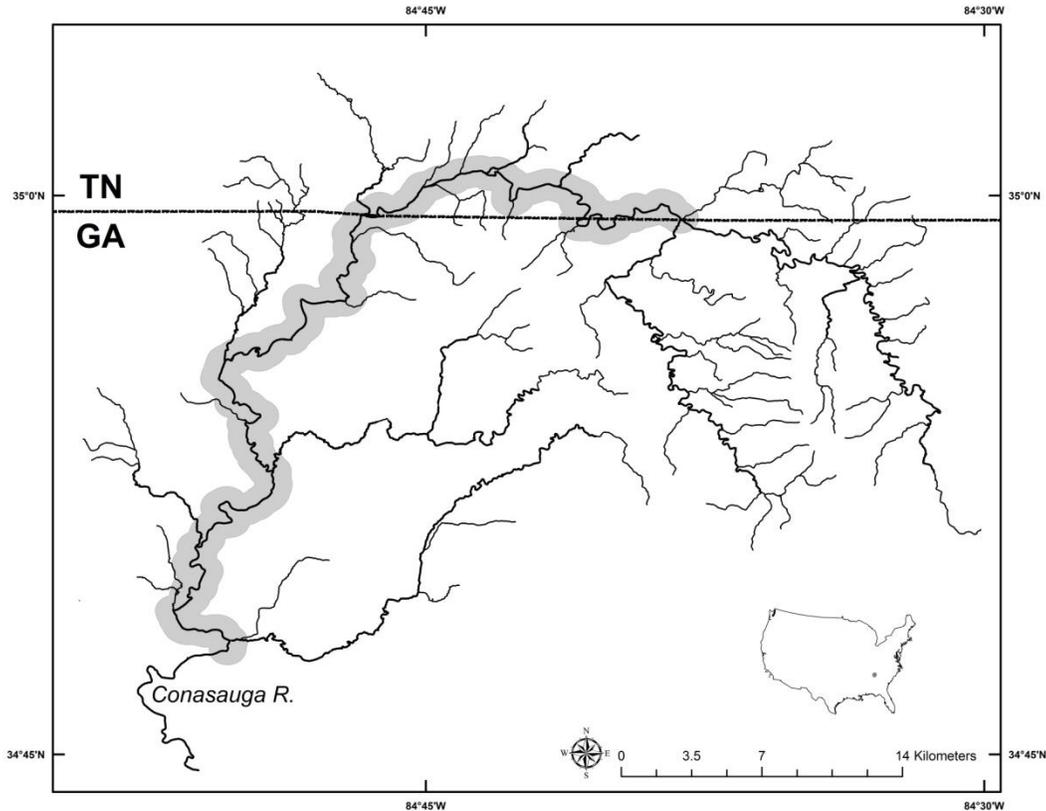


Figure 1. Upper Conasauga River system in Georgia and Tennessee. Known range of *Percina jenkinsi* is shaded in grey. *Percina kathae* used in this study were also collected from this reach.

We used a suite of 12 microsatellite markers known to amplify in *P. rex* (Table 1; Dutton et al. 2008). Polymerase chain reaction (PCR) amplifications were performed in 10 μ L reaction volumes consisting of 30–100 ng of template DNA, 1 \times *Taq* reaction buffer (Applied Biosystems Inc.), 2.00 mM $MgCl_2$, 0.318 mM of each dNTP, 0.25 μ M of each primer, 0.08 U *Taq* polymerase (Applied Biosystems, Inc.). Amplifications were conducted using a GeneAmp PCR system 9700 (Applied Biosystems, Inc.) with the following thermal profile: initial denaturation at 94 $^{\circ}C$ (10 min), followed by a touchdown procedure involving 33 cycles and consisting of denaturing (94 $^{\circ}C$, 30 s), annealing, and extension (74 $^{\circ}C$, 30 s) cycles, where the initial annealing temperature was initiated at 56 $^{\circ}C$ (30 s), and decreased by 0.2 $^{\circ}C$ /cycle. Prior to electrophoresis, 2 μ L of a 1:100 dilution of PCR product was mixed with a 8 μ L solution containing 97% formamide and 3% Genescan LIZ 500 size standard (Applied Biosystems, Inc.). Microsatellite reactions were visualized with an ABI 3130 genetic analyzer (Applied Biosystems, Inc.) using fluorescently labeled forward primers and analyzed using GeneMapper software v4.0 (Applied Biosystems, Inc.).

Table 1. Estimation of *Percina jenkinsi* and *P. kathae* genetic diversity in wild and hatchery broodstock. Abbreviations are total number of sample individuals (N), number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (He).

Taxon (origin)	Locus	N	Na	Ho	He	
<i>P. jenkinsi</i> (wild)	<i>Prex_31</i>	33	4.000	0.545	0.581	
	<i>Prex_32</i>	33	6.000	0.545	0.592	
	<i>Prex_35</i>	33	6.000	0.697	0.669	
	<i>Prex_36</i>	32	1.000	0.000	0.000	
	<i>Prex_37</i>	33	6.000	0.758	0.745	
	<i>Prex_41</i>	33	11.000	0.879	0.837	
	<i>Prex_42</i>	32	11.000	0.906	0.805	
	<i>Prex_43</i>	33	2.000	0.061	0.114	
	<i>Prex_44</i>	32	8.000	0.938	0.834	
	<i>Prex_45</i>	33	7.000	0.788	0.685	
	<i>Prex_46</i>	33	13.000	0.848	0.873	
	<i>Prex_47</i>	33	10.000	0.788	0.868	
	Average			7.083	0.646	0.634
	<i>P. jenkinsi</i> (hatchery)	<i>Prex_31</i>	14	4.000	0.500	0.594
<i>Prex_32</i>		14	5.000	0.571	0.571	
<i>Prex_35</i>		14	5.000	0.500	0.633	
<i>Prex_36</i>		14	1.000	0.000	0.000	
<i>Prex_37</i>		14	6.000	0.714	0.763	
<i>Prex_41</i>		14	10.000	0.929	0.834	
<i>Prex_42</i>		14	9.000	1.000	0.811	
<i>Prex_43</i>		14	2.000	0.071	0.069	
<i>Prex_44</i>		14	7.000	1.000	0.844	
<i>Prex_45</i>		14	6.000	0.857	0.714	
<i>Prex_46</i>		14	10.000	0.857	0.862	
<i>Prex_47</i>		14	8.000	0.929	0.832	
Average				6.083	0.661	0.627
<i>P. kathae</i> (wild)		<i>Prex_31</i>	32	5.000	0.438	0.452
	<i>Prex_32</i>	32	3.000	0.063	0.090	
	<i>Prex_35</i>	31	11.000	0.581	0.738	
	<i>Prex_36</i>	31	13.000	0.774	0.856	
	<i>Prex_37</i>	32	9.000	0.531	0.538	
	<i>Prex_41</i>	31	14.000	0.935	0.908	
	<i>Prex_42</i>	32	8.000	0.750	0.737	
	<i>Prex_43</i>	31	4.000	0.452	0.552	
	<i>Prex_44</i>	0	0.000	0.000	0.000	
	<i>Prex_45</i>	32	13.000	0.844	0.843	
	<i>Prex_46</i>	29	19.000	0.931	0.930	
	<i>Prex_47</i>	31	15.000	0.903	0.877	
	Average			9.500	0.600	0.627

Tests for gametic disequilibrium (all pairs of loci) and locus conformance to Hardy–Weinberg equilibrium (HWE; for each locus in the sampling site) for each taxon were implemented using GENEPOP v4.0.10 (Raymond and Rousset 1995). Significance levels for all simultaneous tests were adjusted using a sequential Bonferroni correction (Rice 1989).

Estimation of genetic diversity, in the form of per locus average number of alleles, observed heterozygosity, and expected heterozygosity were calculated for each taxon using the computer program GenAIEx v6.4 (Peakall and Smouse 2006). We also estimated these parameters for *P. jenkinsi* found in the wild ($n = 33$), collected as brood ($n = 9$), and a random sample of broodstock offspring ($n = 47$) to assess the loss of genetic diversity among these groups.

Effective population sizes (N_e) for *P. jenkinsi* and *P. kathae* samples were estimated using the linkage disequilibrium (LD) method (Hill 1981). The measure of LD was that of Burrow's composite measure (Campton 1987) and was estimated for each species using the program LDNe (Waples and Do 2008). Allele frequencies close to zero can affect estimates of N_e (Waples 2006); therefore, we excluded alleles with frequencies less than 0.02 (Waples and Do 2010). Parametric 95% confidence intervals were also calculated using LDNe (Waples and Do 2008; Waples and Do 2010).

We ran the program BOTTLENECK 1.2.02 (Piry et al. 1999) to test whether samples of *P. jenkinsi* and *P. kathae* underwent a recent bottleneck in genetic diversity. To detect a genetic bottleneck signature we first compared the number of loci that present a heterozygosity excess to the number of such loci expected by chance only (i.e., the sign test). We used the infinite alleles model (IAM) and the two phase model (TPM) under default settings. The allele frequency distribution test was also implemented. The test is a graphical one that examines the frequencies of all alleles in a population and compares this to the distribution expected at mutation-drift equilibrium when rare alleles (i.e. 0.1%) are numerous. When a bottleneck occurs, the expectation is that rare alleles will be lost after the event causing a mode-shift in the distribution of alleles (Luikart et al. 1998).

We used the program STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003) to assess the degree of hybridization between each taxon of interest. The program STRUCTURE was run (using default settings) with three independent replicates for K (i.e., distinct populations or gene pools), with K set to a value of two, representing each species. The burn-in period was 50,000 replicates followed by 500,000 Monte Carlo simulations run under a model that assumed no admixture and independent allele frequencies.

Finally, we evaluated hatchery broodstock contribution via parentage analysis by genotyping broodstock and progeny for five microsatellite markers (Prex_41, 42, 44, 45, and 46). Broodstock consisted of nine individuals that were volitionally tank spawned (note that the sex of each individual was unknown). We randomly sampled 47 offspring from this mating aggregate and matched each parent pair using the program PAPA v2.0 (Duchesne et al. 2002). In doing so, we estimated the number of male and female broodstock as well as the number of progeny produced by each male and female. We used this information to calculate the predicted N_e of the progeny cohort based on the number of male and female broodstock using the equation

$$(1) \quad N_e = \frac{4(N_{male})(N_{female})}{(N_{male}) + (N_{female})}$$

(Wright 1931), where N_{male} and N_{female} were the number of male and female broodstock used to produce hatchery offspring. The predicted N_e assumes that each individual furnished the same number of gametes to the next generation, an assumption that is often violated due to hatchery propagation (i.e., there is typically greater than binomial or Poisson variability in the number of progeny per parent). We thus compared our predicted value to that of observed using information from the number of progeny produced by each parent. Specifically, the observed estimate of N_e was calculated using the equation

$$(2) \quad N_e \approx \frac{Nk - 2}{k - 1 + V/k}$$

(Kimura and Crow 1963), where N was the number of broodstock and k and V were the mean and variance in offspring number. The mean and variance were calculated as

$$(3) \quad \begin{aligned} k &= mk_{male} + (1-m)k_{female} \\ V &= mV_{male} + (1-m)V_{female} + m(m-1)(k_{male} - k_{female})^2 \end{aligned}$$

where m was the proportion of male broodstock and k and V were estimated via parentage analysis.

RESULTS

A total of 33 *P. jenkinsi* and 32 *P. kathae* were collected over the course of the study from the Conasauga River throughout the range of *P. jenkinsi*. Nine of the 33 *P. jenkinsi* individuals were subsequently used as broodstock and a random sample of their offspring ($n = 47$) used to estimate the contribution of hatchery broodstock. All individuals were analyzed using 12 microsatellite markers except for the offspring because preliminary parentage analysis simulations indicated that five microsatellites (Prex_41, 42, 44, 45, and 46) would provide enough genetic information to accurately assign offspring to their respective parents with $> 95\%$ assignment success (data not shown). Also, Prex_44 failed to produce reliable genotype data for *P. kathae*. Both *P. jenkinsi* and *P. kathae* samples were in HWE after correcting for multiple comparisons (all $P > 0.007$ per taxon; $n = 11$ comparisons per taxon for an $\alpha = 0.005$), and each taxon showed no significant evidence of gametic disequilibrium after sequential Bonferroni correction (all $P > 0.009$ per taxon, $n = 66$ comparisons for an $\alpha = 0.0007$).

A comparison of genetic diversity between *P. jenkinsi* and *P. kathae* (Table 1) revealed that the genetic diversity estimates were similar between species. Comparisons of observed and expected heterozygosity between *P. jenkinsi* collected in the wild and those chosen as brood were similar (Table 1); however, 12 of the 85 alleles were lost between wild and broodstock (note that 83% of the alleles were at observed frequencies < 0.05). Using five loci, we were able to compare estimates of genetic diversity of *P. jenkinsi* found in the wild, collected as brood, and a random sample of broodstock offspring. While observed and expected heterozygosity values were similar among comparisons (Table 2), there was a 32% reduction in number of alleles (i.e., a loss of 16 of 50 alleles) due to hatchery influences. Specifically, 12 of the 50 alleles (24% reduction) were lost between wild and hatchery broodstock, with the remainder being lost between hatchery brood and their respective offspring (note that the majority of alleles lost among groups were at observed frequencies < 0.05).

The estimated N_e for *P. jenkinsi* and *P. kathae* was 114 (95% CI 60-526) and -497 (95% CI 264-infinity), respectively. In general, negative estimates indicate that the observed linkage disequilibrium could be explained by sample size alone (Waples and Do 2010), which is the case for a very large population or when the population sample contains too little information. A negative LDNe point estimate is thus uninformative, but the lower bound of the 95% confidence interval can still provide useful information for a lower limit on N_e (Waples and Do 2010).

Table 2. Estimation of *Percina jenkinsi* genetic diversity in wild, hatchery broodstock, and broodstock offspring. Abbreviations are total number of sample individuals (N), number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (He).

Taxon (origin)	Locus	N	Na	Ho	He
<i>P. jenkinsi</i> (wild)	<i>Prex_41</i>	33	11.000	0.879	0.837
	<i>Prex_42</i>	32	11.000	0.906	0.805
	<i>Prex_44</i>	32	8.000	0.938	0.834
	<i>Prex_45</i>	33	7.000	0.788	0.685
	<i>Prex_46</i>	33	13.000	0.848	0.873
	Average			10.000	0.872
<i>P. jenkinsi</i> (broodstock)	<i>Prex_41</i>	9	10.000	1.000	0.833
	<i>Prex_42</i>	9	7.000	1.000	0.796
	<i>Prex_44</i>	9	7.000	1.000	0.827
	<i>Prex_45</i>	9	5.000	0.889	0.704
	<i>Prex_46</i>	9	9.000	0.889	0.846
	Average			7.600	0.956
<i>P. jenkinsi</i> (hatchery offspring)	<i>Prex_41</i>	47	9.000	0.957	0.834
	<i>Prex_42</i>	47	6.000	0.787	0.723
	<i>Prex_44</i>	47	7.000	0.766	0.766
	<i>Prex_45</i>	45	3.000	0.844	0.654
	<i>Prex_46</i>	47	9.000	0.979	0.866
	Average			6.800	0.867

We found no evidence of hybridization between *P. jenkinsi* and *P. kathae* with STRUCTURE results showing that these two taxa appeared genetically distinct (Appendix). Note that one individual (USFWS 841) was identified in the field as *P. kathae*, but genetic analysis indicated that the individual was *P. jenkinsi* (Appendix). We observed no detectable genetic signal for a recent genetic bottleneck in *P. jenkinsi* or *P. kathae*. Sign tests for each taxon reported no significant heterozygosity excess (*P. jenkinsi*, IAM model $P = 0.08$, TMP model $P = 0.51$; *P. kathae*, IAM model $P = 0.53$, TMP model $P = 0.11$). Furthermore, there was no mode-shift detected in allele frequencies for each species.

Results of parentage analysis showed that each male and female contributed offspring but at varying amounts (Table 3). The number of offspring produced by seven males averaged 6.71 and ranged from 1-19. Females produced 18 and 29 offspring, for an average of 23.5 (Table 3). Based on the number of male and female

broodstock, the predicted N_e of the offspring was 6.22. Incorporating the mean and variance in progeny number, the observed N_e (4.97) was less than predicted.

Table 3. Number, mean, and variance of offspring produced from *Percina jenkinsi* broodstock estimated via parentage analysis using five microsatellite markers.

Male ID	# offspring	Female ID	# offspring
2_04	5	2_10	18
2_05	8	2_12	29
2_06	19		
2_07	1		
2_08	11		
2_09	2		
2_13	1		
mean	6.71		23.50
variance	43.57		60.50

DISCUSSION

As expected for randomly mating populations, all loci for sampled *P. jenkinsi* and *P. kathae* were in Hardy Weinberg and linkage equilibria. Genetic diversity estimates based on the average number of alleles and observed heterozygosity for *P. jenkinsi* were somewhat greater than expected for a population with a limited distribution, but values were similar to endangered *P. rex* (Dutton et al. 2008) as well as *P. kathae* (present study). Any genetic signature of a recent bottleneck in genetic diversity went undetected suggesting that if a bottleneck in genetic diversity occurred for *P. jenkinsi*, then it was a more historic rather than a recent event. The increased genetic diversity observed in *P. jenkinsi* could have been attributed to a past hybridization event with *P. kathae* (a closely related and morphologically similar congener); however our results, which were congruent with George et al. (2010), indicated that *P. jenkinsi* and *P. kathae* are distinct taxa with no indication of contemporary hybridization between them.

In an effort to better understand the genetic success of the *P. jenkinsi* breeding program, we assessed the loss of genetic diversity between *P. jenkinsi* in the wild and those used as broodstock, as well as, between broodstock and progeny. Founding of a new population by a small number of individuals (the founder effect; Allendorf 1986) can cause a loss of genetic variation; however, the predicted loss is expected to be different depending on the measure of genetic diversity. Rare alleles are predicted to be especially susceptible to loss; in contrast, heterozygosity should remain relatively unaffected (Allendorf 1986). We found a similar observation for estimates of genetic diversity (i.e., similar heterozygosity levels but

a 24% reduction in the number alleles, inferred from five loci) between *P. jenkinsi* in the wild and those collected as broodstock, suggesting, like others (Hedgecock and Sly 1990, Moyer et al. 2007) that the loss of rare alleles might be a more meaningful measure of genetic change in a hatchery stock than heterozygosity. The observed 32% loss of alleles between the wild *P. jenkinsi* and progeny of hatchery broodstock is alarming, but most of this lost was due to sampling too few broodstock. Fortunately this loss can be mitigated should this program continue in the future by using larger broodstock collections over multiple years (Moyer et al. 2009).

Genetic diversity can also be lost when only a small portion of the broodstock contributes offspring for repatriation in the wild. In an effort to better understand this aspect of genetic success for the *P. jenkinsi* breeding program, we ascertained whether all or only a few broodstock produced offspring for potential stocking. While we were unsure of the actual number of males and females due to difficulties in the identification of each sex, genetic parentage analyses revealed that the broodstock consisted of two females and seven males. All individuals contributed to the gene pool although at varying degrees. Females contributed 18 and 29 offspring with males contributing anywhere from one to nineteen based on a sample of 47 juveniles. If we look at how this spawning success translated to maintenance of genetic diversity (or lack thereof), our observed estimate of genetic diversity (in this case N_e) rivals that of expected indicating little loss of genetic diversity between broodstock and their respective progeny. Thus the hatchery program was successful at minimizing the reduction in N_e between brood and progeny, however, whether there is any further reduction in N_e due family correlated survival (e.g., Moyer et al. 2007) over the lifetime of the offspring remains unknown.

The importance of genetic variation, as a basis for future biological evolution and long-term viability of populations, species, and ecosystems, is well established (Frankel and Soule 1981; Frankham 1995; Hughes et al. 2008). Therefore, identifying and monitoring processes that are likely to have adverse impacts on the conservation of natural populations is an increasingly important endeavor. Unfortunately, most conservation programs do not take full advantage of the potential afforded by molecular genetic markers (Schwartz et al. 2007; Laikre 2010). Genetic data (i.e., N_e , number of alleles, heterozygosity) collected in this study will serve as a reference for comparison in an ongoing effort to monitor temporal changes in population genetic metrics as well as assess and predict potential extinction risks associated with genetic stochasticity. For *P. jenkinsi*, the risk of population decline and extinction due to inbreeding depression and genetic drift appears low. Despite a small contemporary N_e and restricted range, this species

has maintained relatively high levels of heterozygosity and allelic richness. The data presented here also will provide guidance and a means to evaluate the effectiveness (both in terms of increasing the census size and maintaining the long-term viability of the population) of hatchery augmentation in *P. jenkinsi* if the need should arise.

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APPENDIX

STRUCTURE results for classification of *Percina jenkinsi*, *P. kathae*, and potential hybrids. Note that any putative hybrid should have an assignment probability of 0.50 for each taxon. Note individual 841 was identified in the field as *P. kathae*, but molecular analysis identified it as *P. jenkinsi*.

USFWS ID	Taxon ID	Assignment probability		90% probability interval	
		<i>P. kathae</i>	<i>P. jenkinsi</i>	<i>P. kathae</i>	<i>P. jenkinsi</i>
2_01	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.015)	(0.985,1.000)
2_02	<i>P. jenkinsi</i>	0.004	0.996	(0.000,0.016)	(0.984,1.000)
2_03	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
2_04	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
2_05	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.011)	(0.989,1.000)
2_06	<i>P. jenkinsi</i>	0.007	0.993	(0.000,0.048)	(0.952,1.000)
2_07	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.012)	(0.988,1.000)
2_08	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
2_09	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.014)	(0.986,1.000)
2_10	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
2_11	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
2_12	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
2_13	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.009)	(0.991,1.000)
2_14	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.014)	(0.986,1.000)
1703	<i>P. jenkinsi</i>	0.004	0.996	(0.000,0.016)	(0.984,1.000)
1704	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.006)	(0.994,1.000)
1705	<i>P. jenkinsi</i>	0.010	0.990	(0.000,0.074)	(0.926,1.000)
1706	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.013)	(0.987,1.000)
1707	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1708	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1709	<i>P. jenkinsi</i>	0.005	0.995	(0.000,0.029)	(0.971,1.000)
1710	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
1711	<i>P. jenkinsi</i>	0.008	0.992	(0.000,0.051)	(0.949,1.000)
1712	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1713	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.009)	(0.991,1.000)
1714	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1715	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1716	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1717	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1718	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1719	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1720	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1721	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
836	<i>P. kathae</i>	0.998	0.002	(0.994,1.000)	(0.000,0.006)
837	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
838	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
839	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
840	<i>P. kathae</i>	0.993	0.007	(0.952,1.000)	(0.000,0.048)
841	<i>P. kathae</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
842	<i>P. kathae</i>	0.995	0.005	(0.979,1.000)	(0.000,0.021)
843	<i>P. kathae</i>	0.996	0.004	(0.983,1.000)	(0.000,0.017)

844	<i>P. kathae</i>	0.997	0.003	(0.986,1.000)	(0.000,0.014)
845	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
846	<i>P. kathae</i>	0.995	0.005	(0.965,1.000)	(0.000,0.035)
847	<i>P. kathae</i>	0.999	0.001	(0.994,1.000)	(0.000,0.006)
848	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
849	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
850	<i>P. kathae</i>	0.996	0.004	(0.982,1.000)	(0.000,0.018)
851	<i>P. kathae</i>	0.997	0.003	(0.989,1.000)	(0.000,0.011)
852	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
853	<i>P. kathae</i>	0.997	0.003	(0.987,1.000)	(0.000,0.013)
854	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
855	<i>P. kathae</i>	0.994	0.006	(0.969,1.000)	(0.000,0.031)
856	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
857	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
858	<i>P. kathae</i>	0.997	0.003	(0.990,1.000)	(0.000,0.010)
859	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
860	<i>P. kathae</i>	0.993	0.007	(0.962,1.000)	(0.000,0.038)
861	<i>P. kathae</i>	0.998	0.002	(0.990,1.000)	(0.000,0.010)
862	<i>P. kathae</i>	0.998	0.002	(0.990,1.000)	(0.000,0.010)
863	<i>P. kathae</i>	0.990	0.010	(0.924,1.000)	(0.000,0.076)
864	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
865	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
866	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
867	<i>P. kathae</i>	0.997	0.003	(0.987,1.000)	(0.000,0.013)
