# GENETIC MONITORING OF THE RIO GRANDE SILVERY MINNOW: GENETIC STATUS OF WILD AND CAPTIVE STOCKS IN 2020

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Megan J. Osborne and Thomas F. Turner
Department of Biology and Museum of Southwestern Biology MSC 03-2020,
University of New Mexico New Mexico, 87131, USA

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Jen Bachus and Eric Gonzales
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## EXECUTIVE SUMMARY

Genetic monitoring of the middle Rio Grande population of Rio Grande Silvery Minnow (*Hybognathus amarus*) has been conducted annually from 1999-2012 and 2014-2020. This work has included monitoring stocks that were bred or reared in captivity and released to the Rio Grande in New Mexico since 2002; marking the commencement of the augmentation program. In 2020, genetic monitoring was based on genotyping 426 'wild' Rio Grande Silvery Minnow collected from all three occupied reaches of the middle Rio Grande (Table 1 and Table 2), as well as progeny of captive stocks from Southwestern Aquatic Resources and Recovery Center (Southwestern ARRC), Albuquerque Biological Park and the Los Lunas Silvery Minnow Refugium (LLSMR). In 2020, we also genotyped broodstocks held at Southwestern ARRC, Albuquerque Biological Park and the LLSMR.

### *Major findings for 2020*

**(1)** Gene diversity and allelic diversity exceeded 2019 values (Table 3, Figure 1). Average number of alleles (estimated by resampling to account for differences in sample size) increased in 2020, and was equal to the minimum benchmark level for this metric. At the reach level, allelic diversity and gene diversity were virtually identical between the Angostura and San Acacia reaches (sample size was too small for the Isleta reach so we did not calculate diversity statistics) (Figure 2). Likewise, diversity at the reach level was comparable with values seen in 2019, with the exception of heterozygosity, which was higher in 2019 compared to 2020.

**(2)** In 2020, mitochondrial (mtDNA) haplotype diversity and richness increased over 2019 values and were within the range seen across the time series (Table 3, Figure 3). Across all 2020 samples (including hatchery collections) ten haplotypes were detected including two rare haplotypes (I and V) (Table 3); ten haplotypes were also detected in 2018 and 2019. Like previous years, there were no significant differences detected in haplotype frequencies between the three reaches occupied by Rio Grande Silvery Minnow. At the reach level, mtDNA diversity metrics were similar between fish sampled from the Angostura and Isleta reaches (Figure 3). Mitochondrial diversity was lower in the fish from the San Acacia reach in 2020 compared to the Angostura and Isleta reaches.

**(3)** All metrics of genetic effective size increased in 2020 compared with 2019 (Table 2, Figures 4-6). For example, variance genetic effective size estimates for the temporal comparison 2019-2020 ($N_{eV}$=324) increased from the previous temporal comparison 2018-2019 ($N_{eV}$=191). Likewise, linkage disequilibrium effective size ($N_{eD}$=1927) increased from values in 2019 ($N_{eD}$=1329). MtDNA was used to estimate female variance effective population size. For the 2019-2020 temporal comparison, $N_{ef}$ increased from the previous time period and ranged from $N_{ef}$ = 101 - 213 depending on the method used. These results are consistent with the substantial increase in the abundance of Ro Grande Silvery Minnow between 2018 and 2019.

**(4)** Natural recruitment in the Rio Grande in 2019 was strong (Dudley et al. 2020) so fewer individuals were released from hatchery stocks in 2019-2020 compared to 2018 when approximately 200,000 fish were released. To augment the wild population in 2019-2020, 98,790 fish were released to the Rio Grande population; with approximately half released in fall 2019 and the remainder in spring 2020. We genotyped representatives from three captive lots, including one lot from each hatchery facility (Southwestern ARRC, Albuquerque BioPark and LLSMR). Pooled hatchery samples released to the middle Rio Grande in 2019-2020 had levels of gene and allelic diversity measured across microsatellite loci that were very similar to the 'wild' population in 2020, with the exception of observed heterozygosity which was lower in released fish from the Southwestern ARRC and Albuquerque BioPark (Table 2). Across hatchery stocks released in 2019-2020, global haplotype richness and haplotype diversity were lower than in the wild population sampled in 2020 (Table 3).

**(5)** Broodstocks from Southwestern ARCC, Albuquerque BioPark and the Los Lunas Silvery Minnow Refugium (SMR) were also genotyped. These samples represent the 2017, 2018 and 2019 year classes (YC) held at Southwestern ARCC as well as the 2015 and 2018 year classes held at the Albuquerque BioPark, and 2018 year classes from the Los Lunas SMR. Genetic diversity, based on microsatellites and mtDNA, of the broodstocks held at these facilities were within the range seen in the wild population and all 10 haplotypes detected in the wild population in 2020 were present in the broodstock samples. Genetic diversity in the refugial broodstocks will be largely dependent upon the strength of the year class it was from which it was sourced. Genetic effective size estimated using the linkage disequilibrium method ranged from 160 (2017) to infinity (ABP18-4WI) across facilities (Table 2). This estimate pertains to effective size of the generation preceding the current sample.

## INTRODUCTION

Genetic monitoring is defined as a collection of two or more temporally spaced genetic samples from the same population (Schwartz et al. 2007). Such studies typically employ neutral genetic markers and occasionally maternally inherited mtDNA, to track changes in standard genetic diversity metrics (gene diversity [$H_e$], heterozygosity [$H_o$], allelic richness [$A_R$] and genetic effective size [$N_e$]) over a contemporary time series (see glossary). It is widely recognized that erosion of genetic diversity increases a species' vulnerability to decline through lowered fitness (e.g., associated with inbreeding depression) that can ultimately accelerate a species' path to extinction. This is the rationale for tracking these metrics of diversity across time (e.g., Frankham 2005). The time scale of genetic monitoring varies considerably among studies from sampling over only a few years to the use of archival samples for a monitoring program that may span decades. In studies that encompass multiple decades, sampling is rarely conducted on an annual basis so linking changes in diversity metrics with specific environmental or management actions may not be

plausible. In fish, genetic monitoring to date has been confined largely to marine species and freshwater salmonids. The data set that we have collected for Rio Grande Silvery Minnow spans 33 years and represents one of the longest genetic monitoring time series ever collected for a non-salmonid freshwater fish.

For genetic monitoring programs, empirical measurements of diversity and genetic effective size are typically obtained from neutrally evolving microsatellite loci. Microsatellites are short tandemly repeating DNA sequences that are found throughout the genome of most species (reviewed in Dowling et al. 1996). They are bi-parentally inherited and are highly polymorphic among individuals (which is particularly important for endangered species that may have limited genetic diversity) and hence are the most widely used genetic markers in molecular ecology and conservation genetics studies. MtDNA is a haploid marker (i.e., individuals only have one copy as opposed to two copies for microsatellites), so progeny inherit a single mtDNA molecule from the female parent only. Due to differences in how nuclear DNA and mtDNA are inherited, they provide complementary approaches to monitoring genetic diversity.

The endangered Rio Grande Silvery Minnow is a small-bodied (<90 mm standard length), short-lived (in the spring the vast majority of fish are age-1; Horwitz et al. 2018) cyprinid. This species was historically widely distributed in the Rio Grande from northern New Mexico to the Gulf of Mexico, and in the Pecos River from northern New Mexico to the confluence of the Rio Grande in Texas (Pflieger, 1980). Habitat changes associated with river fragmentation caused by water storage dams and diversion structures and changes to the natural hydrograph have resulted in significant range contraction. The interaction of these factors with species life-history causes changes in population density that can exceed an order of magnitude change from one year to the next (Dudley et al. 2020). Over the past 20-years, periodic droughts and resulting channel dewatering have caused recruitment failure in some years and periodic population collapse (Archdeacon et al. 2020a). Today a remnant population persists in the highly fragmented and regulated Rio Grande in New Mexico. This 280-km river segment represents less than 5% of the historical range of the species and extends from downstream of Cochiti Dam to Elephant Butte Reservoir. This stretch of river is bisected by three water diversion structures that define distinct river reaches (from north to south: Angostura, Isleta, and San Acacia). Rio Grande Silvery Minnow was listed under the Endangered Species Act in 1994 (U.S. Department of the Interior 1994). This species is now intensively managed including a captive breeding program and annual augmentation of the Rio Grande population that has been in place since 2003 (Osborne et al. 2012).

The Rio Grande Silvery Minnow population is sampled annually throughout its current range, using nine microsatellite loci and a mtDNA gene to measure the trajectory of genetic diversity measured by allelic richness, heterozygosity, and genetic effective population size. The temporal component and sampling strategy provide the framework necessary to examine impacts of changes in abundance, management actions, and environmental conditions on genetic diversity at these loci.

Negative genetic impacts to a population can occur over relatively short time periods for fishes characterized by a short lifespan (the population is dominated by age-1 fish in the spring; Horwitz et al. 2018) and in which dramatic changes in abundance occur from year to year. As such, genetic monitoring is a crucial component to management of Rio Grande Silvery Minnow. Here, we report on the genetic status of the population in 2020 and compare these results to previous years.

## MATERIALS AND METHODS

*Sampling- Rio Grande population*

Throughout this study, we use the term 'wild' to refer to unmarked fish sampled directly from the Rio Grande, as opposed to individuals tagged with a Visible Implant Elastomer (VIE) tag to indicate that they were reared in a hatchery and used to supplement the Rio Grande Silvery Minnow population. We use the term 'wild caught hatchery' (WCH) to refer to individuals with a VIE tag. 'Wild' fish may have parents that were wild or bred/reared in captivity, but were hatched in the Rio Grande. Unmarked (n=426) Rio Grande Silvery Minnow were collected between October 11$^{th}$ 2019 and March 17$^{th}$ 2020; these are assumed to represent the potential breeding population in 2020. These samples add to the data collected from wild Rio Grande Silvery Minnow sampled from the middle Rio Grande annually from 1999 to 2012 and 2014-2019 (between November and April- just prior to reproduction) as well as 43 individuals used in a previous allozyme study of *Hybognathus* and stored in the Museum of Southwestern Biology Division of Genomic Resources (Cook et al. 1992 - referred to as 1987 sample). The distinction is made between 'wild' and WCH fish for this reason and because population monitoring tracks 'wild' fish separately from hatchery released fish. Collections were made throughout the current distribution (i.e., from Cochiti reservoir to Elephant Butte reservoir in New Mexico) of Rio Grande Silvery Minnow, with the exception of the Cochiti reach because the species is rare or absent in that area (Bestgen and Platania 1991). In 2020, wild fish were collected from all occupied river reaches by seining a variety of habitats in the Angostura (n=148), Isleta (n=127) and San Acacia (n=151) reaches (Table 1, Table 2). Fish were anesthetized in river water treated with MS-222 (Tricaine methane sulfonate 200 mg/L river water) at the site of capture. A piece of caudal fin was removed from each individual. Fin clips were preserved in 95% ethanol prior to isolating DNA.

*Sampling- Captive lots and Broodstock*

In 2020, fin clips representing fish released in fall 2019 and spring 2020 from Southwestern ARRC (n=147), Albuquerque BioPark (n=50) and LLSMR (n=101; referred to as Los Lunas released) were genotyped. We also genotyped broodstocks held at Southwestern ARRC (2017 n=99; 2018 n=100; 2019 n=96), Albuquerque Biological Park (2015 n=100; 2018 n=99) and the LLSMR (2018 n=138; lot id ABP18-4WI).

*Molecular methods- microsatellites*

Total nucleic acids, including genomic and mitochondrial DNA were extracted from air-dried fin clips using proteinase-K digestion and organic extraction methods (Hillis et al. 1996). Individuals were genotyped at nine microsatellite loci: *Lco1*, *Lco3*, *Lco6*, *Lco7*, *Lco8* (Turner et al. 2004); *Ca6* and *Ca8* (Dimsoski et al. 2000); and *Ppro118* and *Ppro126* (Bessert and Orti 2003). The following pairs of loci were amplified through multiplex PCR: *Lco1*/*Ca6* and *Lco6*/*Lco7* (1X PCR buffer, 3 mM $MgCl_2$, 125 micromol [μM] deoxyribonucleotide triphosphates [dNTPs], 0.40-0.50 μM each primer, 0.375 units *Taq* polymerase); *Lco3* and *Lco8* (1X PCR buffer, 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.40-0.50 μM each primer, 0.375 units *Taq*); and *Ppro 118*/*Ppro126* (1X PCR buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.40-0.50 μM each primer, 0.375 units *Taq*). *Ca8* was amplified alone (1X PCR buffer, 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.50μM each primer, 0.375 units *Taq* polymerase). PCR cycling conditions for all loci were as follows: one denaturation cycle of 92°C for 2 min followed by 30 cycles of 90 °C for 20s, 50°C for 20 s, 72°C for 30s. Cycling conditions for *Ppro 118*/*Ppro126* were as follows: one denaturation cycle of 92°C for 2 min followed by 30 cycles of 90 °C for 20s, 60°C for 20 s, 72°C for 30s. Primer concentrations in multiplex reactions were optimized by locus to ensure equal amplification each microsatellite. Fragment size analysis on an ABI 3130 automated capillary sequencer was performed by combining 1 μl of PCR product with 10 μl of formamide and 0.4 μl of HD400 size standard and denatured at 93°C for 5 minutes. Genotype data were scored in GENEMAPPER Version 4.0 (Applied Biosystems).

*MtDNA- ND4*

A 295-base pair (bp) fragment of the mtDNA ND4 gene was amplified from each individual in a 10 μL reaction containing 1 μL template DNA, 1 μL 10× reaction buffer, 2 mM $MgCl_2$, 0.8 μM dNTPs, 0.5 μM forward (5'- GAC CGT CTG CAA AAC CTT AA- 3') and reverse primer (5'- GGG GAT GAG AGT GGC TTC AA – 3'), and 0.375 units *Taq*. PCR conditions were 90° C initial denaturation for 2 minutes followed by 30 cycles of 90° C for 30 seconds, 50° C for 30 seconds, and 72◦ C for 30 seconds. Sequence data was obtained for all individuals by direct sanger sequencing (Big Dye vers. 1.1) according to the manufacturer's instructions and using an ABI 3130 DNA Sequencer.

*Statistical analysis*

GENEPOP'007 (Rousett 2008) was used to test for departures from Hardy-Weinberg equilibrium (HWE), using the procedure of Guo and Thompson (1992) and to perform global tests for linkage disequilibrium for all pairs of loci in each collection. Sequential Bonferroni correction (Rice 1989) was applied to account for inflated Type-1 error rates associated with multiple simultaneous tests. In some cases, sample sizes differed between collections, particularly between some samples collected early in the study and more recent collections. The number of alleles and heterozygosity are dependent on sample size, so we used a resampling approach to correct for sample size effects on diversity measures and make them more comparable across collections. In short, we randomly sampled each collection without replacement using the minimum sample size across all years (n =

43 in 1987). Microsatellite diversity estimates (corrected number of alleles [$N_{AC}$], Nei's unbiased gene diversity (Nei 1987) [$H_{EC}$] and heterozygosity [observed proportion of heterozygotes] [$H_{OC}$]) were then calculated for the random sample and the process repeated for 1000 iterations. Corrected diversity estimates are calculated as the mean estimate across all iterations. This analysis was conducted in the R statistical software package (www.r-project.org). This resampling technique was also used for comparisons among collections obtained across years and river reaches, we repeated the resampling procedure for microsatellite data with diversity measures based on n=15 (1987, San Acacia) and excluding the smallest samples (2004 San Acacia; Isleta 2019). For each microsatellite locus and population, inbreeding coefficients ($F_{IS}$) were obtained using the R statistical package *hierfstat* (Goudet and Jombart 2015).

Mitochondrial diversity was characterized by number of haplotypes ($N_h$), haplotype diversity (*h*), and haplotype richness ($H_R$). These metrics are roughly equivalent to the number of alleles, gene diversity ($H_{EC}$), allelic diversity ($N_{AC}$) averaged across microsatellite loci. Haplotype richness ($H_R$) was obtained using the program CONTRIB vers.1.02 (Petit et al. 1998) which uses a rarefaction approach to correct for unequal sample sizes. Haplotype diversity (*h*) is a measure of the uniqueness of a haplotype in a population. Values of *h* range from zero (all individuals have the same haplotype) to one (all individuals have a different haplotype). The calculation of *h* is based on the sample size and the frequency of each haplotype in the population.

To place levels of diversity across years in context of overall genetic diversity of the species and to develop a biologically relevant benchmark for assessing levels of diversity within samples, we used an additional resampling technique. All 'wild' fish were pooled into one large population (n = 6407) from which we iteratively took samples (n = 43 by year; n=15 by reach benchmarks) to estimate diversity statistics. Our primary interest is maintaining genetic diversity, hence we estimated a one-tailed lower 95% confidence intervals that corresponds to the upper 95% of the resampled distribution (i.e., 9500 of 10000 iterations). Thus, the distribution contained within this confidence interval corresponds to the null hypothesis of no loss of diversity.

*F-statistics*

Weir and Cockerham's (1984) *F*-statistics (microsatellites) and *Φ*-statistics (mtDNA) were calculated in Arlequin ver. 3.5 (Excoffier and Lischer 2010). Hierarchical analysis of molecular variance (AMOVA) was used to test whether a significant proportion of genetic variance was partitioned into components attributable to differences among 'wild', captive-spawned, and broodstock fish ($F_{CT}$, $\Phi_{CT}$), among samples within these three groups sampled in 2019 ($F_{SC}$, $\Phi_{SC}$) and among all samples ($F_{ST}$, $\Phi_{ST}$). P-values for all statistics were generated using bootstrapping (1000 permutations), as implemented in the program Arlequin. We also calculated pairwise $F_{ST}$ values between all temporal samples collected from the Rio Grande and obtained upper and lower 95% CIs using boostrapping (1000 permutations) implemented in the R package diversity vers 1.9 (Keenan 2017).

*Estimation of genetic effective size*

Variance effective size ($N_{eV}$) and 95% confidence intervals (CIs) were estimated from temporal (annual) changes in microsatellite allele frequencies across annual samples, using the temporal method (Nei and Tajima 1981; Waples 1989) implemented in NEESTIMATOR (Do et al. 2014). Highly polymorphic loci with many rare alleles, as is typical of microsatellites, can be subject to biased estimates of variance effective size, $N_{eV}$, (Hedrick 1999; Turner et al. 2001). To account for this potential bias, the unbiased estimator, $F_S$, (Jorde and Ryman 2007), as implemented in NEESTIMATOR, was also used to estimate $N_{eV}$. Rio Grande Silvery Minnow were sampled under Plan I (prior to reproduction, with replacement) for all methods. Jackknife estimation (sequentially dropping each locus and re-estimating the statistic on the reduced dataset) over all loci was used to calculate $N_{eV}$ and associated 95% confidence intervals. Multiple temporal methods are used to calculated $N_{eV}$ to ensure consistency across estimators. Additionally, $N_{eV}$ was estimated using the pseudo-maximum-likelihood (MLNE) method implemented in the program MLNE (Wang and Whitlock 2003).

For all methods, we assumed that immigration (except from hatchery stocks) from outside the study area did not affect estimates of $N_e$. We equated the number of years separating a pair of samples with the number of generations elapsed between samples because Rio Grande Silvery Minnow have essentially non-overlapping generations (based on unpublished population monitoring data of R. K. Dudley and S. P. Platania). However, to account for small but known deviation from the discrete generation model (G = 1.27), we corrected consecutive estimates of $N_{eV}$ and $N_{ef}$ for overlapping generations (Turner et al. 2006; Osborne et al. 2010), using the analytical method of Jorde and Ryman (1995, 1996). In addition to consecutive pairwise estimates, we also present comparisons between the 1987 and 1999 samples to provide historical context for the contemporary estimates. As these samples (1987-1999) were collected more than 3-5 generations apart, the drift signal should be sufficiently large relative to sampling biases associated with age-structure such that correction for overlapping generations is unnecessary (Waples and Yokota 2007).

In addition to estimates of $N_{eV}$ that require samples from different time periods, we used the linkage disequilibrium method ($N_{eD}$, Hill 1981) that only requires a single temporal sample. Annual $N_{eD}$ was estimated from microsatellite DNA data separately for 'wild', WCH and captive-spawned stocks using the program NEESTIMATOR (Do et al. 2014) and methods described in Osborne et al. (2012). Single sample $N_e$ methods (such as those provided by $N_{eD}$) yield an estimate of the effective number of parents that produced the progeny from which the sample is drawn, and most closely approximates the inbreeding effective size, $N_{eI}$ (Laurie-Ahlberg and Weir 1979; Waples 2005).

Variance effective size was also estimated for the female portion of the population using mtDNA haplotype frequencies. To distinguish between variance effective size based on microsatellite data ($N_{ev}$) we use the designation $N_{ef}$ to represent mtDNA variance effective size. $N_{ef}$ was estimated with temporal (aka 'moments' method) and pseudo-maximum-likelihood (MLNE) methods (Wang

2001). It is useful to estimate genetic effective size from mtDNA data because it provides information pertaining to the female portion of the population. For example, if very low estimates of $N_{ef}$ were obtained it would suggest that few females are making a genetic contribution to the population.

## RESULTS

*Microsatellites- genetic diversity*

Characterization of microsatellite genotypes from the 2020 samples revealed two loci (*Ca6* and *Ppro126*) as the least variable, each with 9 and10 alleles, respectively, detected across samples. Locus *Ppro118* was the most variable with 60 alleles followed by *Lco1* with 39 alleles detected across 2019 collections. In 2020, tests for deviations from Hardy-Weinberg Proportions were significant for 37% of locus-by-site combinations (37/90) following sequential Bonferroni correction for multiple comparisons. The majority of significant departures occurred at four loci (*Lco7*, *Lco8*, *Ca8*, *Ppro118*). Analysis by MICRO-CHECKER (Van Oosterhout et al. 2004) found that presence of null alleles was the most likely explanation for an excess of homozygous individuals. Three loci (*Lco3*, *Lco6*, *Ca6*) conformed to Hardy-Weinberg expectations in all collections while *Ppro126* (Dx YC19), *Lco1* (DxYC19, SNARRC Bs YC18) and *Ca8* departed expectations in a few collections each. Across all collections, significant tests for genotypic disequilibrium occurred in 6 of 359 comparisons following sequential Bonferroni correction; all significant tests occurred in two captive stocks (Los Lunas released and SNARCC Bs YC17).

Genetic diversity statistics based on microsatellite data of 'wild' Rio Grande Silvery Minnow sampled for 2020 ($H_{EC}$= 0.813, $H_{OC}$ = 0.729, and $N_{AC}$= 14.7) were within the range of values observed since monitoring began (Table 3, Fig 1). The lowest number of alleles and heterozygosity were observed in the 1999 sample ($N_{AC}$ = 12.1, $H_{OC}$ = 0.65) and lowest gene diversity ($H_{EC}$= 0.782) was recorded in 2002; prior to the commencement of population augmentation. The greatest number of alleles were observed in 2012 ($N_{AC}$ = 15.2), gene diversity was greatest in the 2007 sample ($H_{EC}$= 0.84) and heterozygosity was highest in 2004 ($H_{OC}$ = 0.74).

We used a resampling approach of all 'wild' fish collected between 1987-2020 to determine diversity benchmarks that correspond to annual diversity estimates based on the minimum annual sample size (n = 43 [year], n=15 [reach]). Benchmarks were obtained for microsatellite diversity estimates ($H_{EC}$ = 0.80, $H_{OC}$ = 0.68, and $N_{AC}$= 14.7 for annual samples and $H_{EC}$ = 0.766, $H_{OC}$= 0.648, and $N_{AC}$= 9.6 at the reach level) (Fig 1). Observed levels of diversity for 2020 'wild' fish exceeded these benchmarks. From analysis of microsatellite data by river reach all diversity metrics for the 2020 sample exceeded the minimum benchmark values. Across all captive stocks genotyped in 2020, gene diversity and heterozygosity for pooled captive lots released to the Rio Grande in fall 2018 and spring 2019 were greater than the lower 95% CI genetic diversity benchmark (Table 3). However, allelic diversity from the pooled captive lots released in fall 2019 and spring 2020 ($N_{AC}$ =

14.58) as well as individual stocks fell below the benchmark estimate. Likewise, gene diversity and heterozygosity fell below the benchmark estimates in one captive stock released in fall 2019-spring 2020 (DX_YC19). This result may be an artefact of more missing data than normal in this collection; a results of poor quality DNA. All measures of genetic diversity in the refugial broodstocks sampled in 2020 from the three facilities, were very similar to estimates in the middle Rio Grande population (Table 3).

*MtDNA- genetic diversity*

A total of 17 mtDNA haplotypes have been identified from assaying > 6000 (untagged) individuals from the middle Rio Grande from 1987 to 2020 (Table 4). Haplotype A was the most common in almost all samples including the 2020 collection. In the 2020 'wild' population, haplotype A was present in 48% of individuals, haplotypes (C, D, F, O) were present at moderate frequencies (6-20% of individuals) and five haplotypes (E, I, K, M and V) were uncommon (<5%). Across the time series, haplotype diversity was highest in the 1987, 2016 and 2018 samples and lowest in 2000 ($h$ = 0.392) (Table 3, Figure 3). In 2020, haplotype diversity ranged from 0.707 ('wild') to 0.50 (ABQ BioPark Bs YC15). Both haplotype diversity and richness observed in 2020 'wild' samples increased over values in 2019 (Figure 3; Table 3). In 2020, two rare haplotypes (I and V) were detected in a small number of wild and hatchery individuals. Six haplotypes were observed in the BioPark broodstock (2015 and 2018 YC), 8-9 haplotypes were observed in the Southwestern ARRC broodstocks (2017, 2018 and 2019 YC) and 10 haplotypes were detected in the Los Lunas Silvery Minnow Refugium broodstock which originated from wild caught individual collected in 2018 and reared at the Albuquerque BioPark (Table 5). When comparing mitochondrial diversity among broodstocks, diversity was reduced in the Southwestern ARRC 2015 year-class. This is not surprising given this sample represents the wild population immediately following the population bottleneck that occurred from 2012 to 2014 (Dudley et al. 2020).

Haplotype diversity and haplotype richness were higher in the Isleta reach ($h$= 0.744, $H_R$= 3.53) compared to the San Acacia reach ($h$=0.681, $H_R$= 3.26) in 2020. At the reach level in 2020, 9-10 haplotypes were detected and mtDNA diversity statistics increased over 2019 values (Figure 3).

*Population structure- microsatellites*

Total population structure was evaluated by considering global $F_{ST}$ estimates across all 2020 samples, including hatchery stocks and broodstock (Table 6). Samples from the middle Rio Grande collected in 2020 differed significantly from almost all captive stocks with values of $F_{ST}$ ranging from 0.008 to 0.014. The 2019 broodstock sample held at the Albuquerque BioPark and the 2019 wild sample were not significantly different from the 2020 wild sample (recruits from 2019). There were also significant differences among most captive stocks. In 2020, there were no significant differences between Rio Grande Silvery Minnow collected in the three river reaches ($F_{ST}$=0.002, p-value=0.657 between Angostura and Isleta, $F_{ST}$=0.002, p-value=0.014 between Angostura and San Acacia, and $F_{ST}$=0.001, p-value=0.097 between Isleta and San Acacia) following Bonferroni

correction. Results of the AMOVA indicated that there was significant variance of allele frequencies among samples within groups ($F_{SC}$ = 0.011, p-value=0.00001) and among groups (wild, released captive fish and broodstock) ($F_{CT}$=0.005, p-value=0.011). The highest pairwise $F_{ST}$ between temporal samples collected from the Rio Grande was 0.017 (2002 vs 2007). Average pairwise $F_{ST}$ between 1987 and 2012 was 0.004 and between 2015 and 2020 average $F_{ST}$ was 0.0007 (Table 8).

*Population structure- mtDNA*

Total population structure was evaluated using global $\Phi_{ST}$ estimates across all 2020 samples, including hatchery stocks and broodstock (Table 7) based on mtDNA haplotype frequencies. We also included the 2019 sample collected from the middle Rio Grande population. After Bonferroni correction, there were 11 significant pairwise comparisons, all of which involved two captive stocks (DxYC_19 and Los Lunas released). Φ-statistics were also calculated from mtDNA data across all wild-caught Rio Grande Silvery Minnow across the time-series (1987, 1999-2020). Genetic differences among the Angostura, Isleta, and San Acacia reaches were not significant across the time series ($\Phi_{CT}$ = 0.0006, P = 0.169). Likewise, pairwise $\Phi_{ST}$ between reaches in 2020 were not significantly different from zero ($\Phi_{ST}$=0.009, p-value=0.144 between Angostura and Isleta; $\Phi_{ST}$=0.009, p-value=0.065 between Angostura and San Acacia, $\Phi_{ST}$= 0.0009, p-value=0.298 between Isleta and San Acacia).

*Genetic effective size*

Temporal and MLNE estimates of variance effective size, $N_{eV}$, from microsatellites, are shown in Figure 4. For 2019-2020, estimates of $N_{eV}$ across methods ranged from 324-844; an increase from the previous time period (2018-2019). All $N_{eV}$ estimates had finite upper and lower 95% CIs. MLNE and temporal estimates of female variance effective size, $N_{ef}$, based on mtDNA are shown in Figure 5. For 2019-2020 temporal comparison, estimates of $N_{ef}$ increased from the previous time period to $N_{ef}$ = 101 (95% CI 13-infinity) for the temporal method and $N_{ef}$ = 213 (95% CI 39-infinity) calculated using the maximum likelihood method.

Inbreeding effective size (Figure 6; Table 2) was $N_{eD}$ = 1927 (95% CI 1131-5627) for wild fish collected in 2020; an increase from the 2019 estimate. For captive stocks released in the middle Rio Grande in fall 2019 and spring 2020 from Albuquerque BioPark, $N_{eD}$ = 78. Effective size of captive stocks from Southwestern ARRC was $N_{eD}$ = 1056 and from the Los Lunas released $N_{eD}$=62.

Estimates of $N_{eD}$ of refugial broodstock held at the Albuquerque BioPark were $N_{eD}$ = 384 (YC 2015) and $N_{eD}$ = 591 (YC 2018), Los Lunas SMR (ABP18-4WI) was $N_{eD}$ = infinite and for Southwestern ARRC $N_{eD}$ = 145 (YC 2017), 477 (YC 2018) and 495 (YC 2019). These values reflect the effective size of the generation preceding the broodstock samples.

## DISCUSSION

*Genetic monitoring*

Monitoring genetic diversity parameters ($H_E$, $H_O$, $A_R$ and $N_e$) across contemporary time-series can illuminate demographic and evolutionary processes affecting wild and captive populations that are unattainable using standard demographic sampling approaches. To our knowledge, data from Rio Grande Silvery Minnow is one of the longest genetic monitoring time-series for any non-salmonid freshwater fish; spanning 33 years (1987 - 2020). Annual monitoring of the genetic status of both 'wild' Rio Grande Silvery Minnow in the middle Rio Grande in addition to representative captive stocks repatriated to the river allows assessment of whether management actions are maintaining levels of genetic diversity in the species. Maintenance of diversity is critical because genetic diversity facilitates adaptation and responses to changing conditions.

*Status of the 'wild' (i.e. untagged) Rio Grande Silvery Minnow population in 2020*

The population monitoring program for Rio Grande Silvery Minnow (1993-2020) shows that the wild population has experienced multiple, order-of-magnitude changes in density over the past two decades (Dudley et al. 2020). The period from 2015-2017 saw abundances of Rio Grande Silvery Minnow increase substantially over values recorded between 2012-2014, demonstrating the capacity of the species to rebound rapidly following periods of very low density. Subsequently, there was a 99.6 % decline in estimated density between 2017 and 2018 (Dudley et al. 2020) associated with extensive channel drying (> 60 km) between April through October 2018 and almost complete recruitment failure (Archdeacon et al. 2020a). This occurred as a result of a period of moderate to severe drought that extended from December 2017 to February 2019 (Archdeacon et al. 2020a\). In 2019, the population increased 2,285% over densities seen in the previous year associated with extended high spring flows and minimal channel drying and strong recruitment (Dudley et al. 2020; Archdeacon et al. 2020). Repeated changes in population size particularly repeated bottlenecks are expected to gradually erode genetic diversity particularly in the absence of actions to buffer the population (i.e. supportive breeding and augmentation). From 1987 and 1999-2004, both microsatellites and mtDNA showed considerable inter-annual variability in gene diversity metrics and effective size estimates. Following commencement of population supplementation with fish reared in captivity, inter-annual variability in diversity measures decreased from 2005 to 2012 and during this period there were marginal increases in mtDNA and microsatellite diversity. Since 2015, genetic diversity has remained fairly stable despite the significant population bottleneck in 2018. These results demonstrate that the augmentation program has been critical to maintenance of diversity in the face of repeated population bottlenecks.

*Genetic effective size*

Genetic effective population size ($N_e$) is a key parameter in genetic monitoring programs because $N_e$ determines the amount of variation that is transmitted to the next generation. Specifically, the rate at which diversity is lost is inversely proportional to the effective size (i.e., at smaller $N_e$,

diversity is lost more rapidly). In 2020, genetic effective size estimates ($N_{eV}$, $N_{eD}$, and $N_{ef}$) increased from the most recent temporal estimates. An increase in $N_{eV}$ is indicative of reduced drift in allele frequencies between 2019 and 2020 than for the previous temporal comparison (2018-2019). Estimates of female effective size made from mtDNA haplotype frequency data indicated increased maternal contributions compared to the previous time period; consistent with the substantial increase in population size in 2019 compared to 2018.

Genetic effective size estimated for the middle Rio Grande population (untagged fish) using the linkage disequilibrium method, also increased over the 2019 estimate. This method is a single sample estimator and uses different aspects of the data to estimate the effective size. From a management perspective, there are a number of theoretical and practical distinctions between $N_{eI}$ (inbreeding effective size, to which $N_{eD}$ estimates are most closely associated) and $N_{eV}$ (variance effective size). These two measures of effective size should be similar in stable populations but show predictable differences in declining (or growing) populations. For example, in declining populations $N_{eI}$ should be larger than $N_{eV}$ because the latter depends on the amount of genetic drift between sampled generations but the former is a measure of inbreeding in the generation prior to sampling, (Allendorf & Luikart 2007); therefore, $N_{eI}$ is only reduced once mating between close relatives becomes more common (i.e., homozygosity increases in the population). In previous years, we observed a disparity between $N_{eD}$ and $N_{eV}$ and this trend continued in 2020. Carson et al. (2020) investigated the relationship between $N_{eD}$ and $N_{eV}$ using simulations and showed that the disparity between $N_{eV}$ and $N_{eD}$ is driven by the interaction of population augmentation and fragmentation. Specifically, when upstream dispersal is disrupted by barriers (e.g., dams) $N_{eI}$ is negatively associated with the rate of downstream dispersal (i.e., $N_{eI}$ declines with higher rates of downstream movement). $N_{eI}$ is positively associated with supplementation rate (i.e., $N_{eI}$ increases with higher rates of supplementation). In contrast, $N_{eV}$, is negatively associated with both bidirectional dispersal rates and supplementation relative to equilibrium $N_e$.

*Genetic diversity of captive stocks released to the middle Rio Grande, New Mexico*

In fall 2019 and spring 2020, approximately 100,000 fish were released in the middle Rio Grande, New Mexico. Microsatellite diversity statistics for the pooled hatchery samples had values marginally lower than the benchmark values but that were almost identical to the "wild" population sampled in 2020. In 2020, mitochondrial haplotype richness (corrected for differences in sample size) was fairly consistent among captive lots and facilities. Preservation of genetic diversity in captive lots is critical because these fish periodically reestablish the species in the middle Rio Grande (e.g., 2014 and 2018).

*Genetic Analysis of Broodstock*

In 2020, we genotyped fish representing the 2015, 2017, 2018 and 2019 broodstock year classes. Allelic diversity was marginally lower than the benchmark value while gene diversity and heterozygosity generally exceeded these benchmarks. Diversity in the 2015 samples was lower than

in the more recent year classes and this is not surprising as the 2015 sample represents a post-bottleneck sample. Diversity is expected to be reduced following a multi-year bottleneck. The diversity in the 2015 is presumably reflective of the samples that reproduced in 2014; a year of extremely low recruitment and heavy augmentation with captive reared fish. These results highlight the continued importance of spawning large numbers of individuals to preserve rare alleles.

## CONCLUSIONS

Twenty years of genetic monitoring of the 'wild' middle Rio Grande population and of released captive reared/bred Silvery Minnow provides a rare opportunity to track the genetic effects of population fluctuations associated with inter-annual variability in flows and of various management activities. The results presented here indicate that the trajectory of genetic change in the wild Rio Grande Silvery Minnow population is now determined largely by supplementation with captive reared stocks. Supplementation buffers the population against potential losses of diversity predicted by drastic changes in population size (Osborne et al. 2012). Levels of genetic diversity including heterozygosity and average number of alleles have so far been maintained over the duration of the study. This highlights the importance of continued monitoring of both the captive stocks and the wild population as any detrimental effects (such as losses of diversity) in the captive stocks will ultimately be transferred to the 'wild' population.

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## GLOSSARY

**Allelic richness –** The total number of alleles in a population corrected by rarefaction to account for differences in sample size among collections.

**Bottleneck –** a population or genetic bottleneck is the dramatic reduction in population size typically associated with an environmental/demographic event. Genetic drift that results from a bottleneck reduces the amount of genetic diversity in generations following the event. This diversity can only be recovered by mutation or by gene flow from another population or from captive populations.

**Genetic drift –** is the random change in allele frequencies from generation to generation because of sampling error. Specifically, the finite number of genes passed on to progeny will be an imperfect sample of the parental allele frequencies. The effects of genetic drift are (i) allele frequencies will change and (ii) genetic variation will be lost. The smaller the population, the greater the change in allele frequencies due to drift.

**Genetic effective size ($N_e$) –** The effective size of a breeding population under idealized conditions meeting the assumptions of Hardy-Weinberg (i.e., equal sex ratio, random mating).

**Haplotype/ gene diversity (*h*) –** Computationally equivalent to expected heterozygosity ($H_e$) but referred to as gene diversity as there are no heterozygotes because mtDNA is haploid.

**Hardy-Weinberg equilibrium –** The stable frequency distribution of genotypes (AA, Aa, and aa) in the proportions ($p^2$, 2pq, and $q^2$) respectively (where p and q are the frequencies of the alleles, A and a). The Hardy-Weinberg principle makes the following assumptions (i) random mating (i.e. there is neither preference or aversion), (ii) no mutation (i.e. genetic information is transmitted from parent to progeny without change), (iii) large or infinite population size, (iv) no natural selection, (v) no immigration.

**Heterozygosity ($H_e$) –** The presence of different alleles at one or more loci on homologous chromosomes. Proportion of heterozygous individuals for a locus in a population.

**Inbreeding co-efficient (F) –** the probability that two alleles at a locus in an individual are identical by descent. Used to measure the extent of inbreeding.

**Linkage disequilibrium –** statistical association of alleles at different loci. Such association indicates that two loci are physically adjacent on a chromosome such that there is little recombination during meiosis.

**Locus/Loci –** A segment of DNA on a chromosome. Loci is the plural form of the noun.

**Microsatellite –** short tandem repeated DNA sequences e.g. ACACACAC. These loci usually have

variable numbers of repeats within/among individuals and high heterozygosity.

**Mitochondrial (mt) DNA –** maternally inherited circular DNA molecule contained within the mitochondria.

**Null allele –** a mutation that occurs in a PCR primer site that prevents amplification during polymerase chain reaction (PCR).

**Primers –** short fragments of DNA that flank the DNA region of interest and which are used in PCR to target specific nuclear and mtDNA loci.

**Polymerase chain reaction –** method used to make copies through amplification of a specific segment of DNA (such as a microsatellite locus or mitochondrial DNA gene). DNA is heated in the presence of PCR primers, and the Taq polymerase enzyme, to copy the intervening DNA sequencing using ~30 cycles.

**Ryman-Laikre effect –** an increase in inbreeding and reduction in the total effective population size that can occur in wild-captive systems that occurs when few individuals contribute large numbers of offspring.

**SNP (single nucleotide polymorphism**) **–** a nucleotide position that varies among individuals in a population.

**Wahlund effect –** is a reduction in heterozygosity compared to Hardy-Weinberg expectations, and occurs in a population divided into partially isolated subpopulations

**'Wild' vs. 'captive' –** we use the term 'wild' to refer to unmarked fish sampled directly from the Rio Grande. 'Wild' fish may have parents that were wild or bred/reared in captivity, but were hatched in the Rio Grande. The term 'captive' refers to a fish held in a hatchery or a VIE-tagged fish captured from the Rio Grande.

**Table 1.** Sample sizes and collection localities of wild Rio Grande Silvery Minnow by river reach for samples collected during 2020 genetic monitoring.

| | |
|---|---|
| Angostura | 148 |
| Montano Bridge Crossing | 49 |
| Central Avenue bridge crossing (US HWY 66) | 48 |
| Avenida Cesar Chavez Bridge Crossing | 51 |
| Isleta | 127 |
| 346 Bridge Crossing | 28 |
| Los Lunas Bridge crossing (NM State HWY 49), Los Lunas | 52 |
| ca. 3.7 mi downstream of US HWY 60 bridge crossing, Bernardo | 47 |
| San Acacia | 151 |
| Escondida | 51 |
| ca. 1.5 mi downstream of San Acacia Diversion Dam | 51 |
| San Marcial Railroad Bridge | 49 |
| **Grand Total** | 426 |

**Table 2.** All 'wild' samples collected from the middle Rio Grande by river reach for 1987, 1999 -- 2012 and 2014 -- 2020. *Genetic analysis was not conducted in 2004 due to the small number of samples collected.

| Year | Angostura | Isleta | San Acacia | Total |
|---|---|---|---|---|
| 1987 | 15 | - | 28 | 43 |
| 1999 | - | - | 46 | 46 |
| 2000 | - | - | 194 | 194 |
| 2001 | - | 65 | 63 | 128 |
| 2002 | 67 | 121 | 201 | 389 |
| 2003 | 71 | 65 | 33 | 169 |
| 2004 | 141 | 15 | 6 | 162 |
| 2005 | 190 | 109 | 95 | 394 |
| 2006 | 95 | 143 | 145 | 383 |
| 2007 | 48 | 128 | 42 | 218 |
| 2008 | 165 | 191 | 123 | 479 |
| 2009 | 175 | 153 | 150 | 478 |
| 2010 | 149 | 146 | 151 | 446 |
| 2011 | 71 | 148 | 140 | 359 |
| 2012 | 147 | 215 | 154 | 516 |
| 2013 | - | - | - | - |
| 2014* | 5 | 3 | 4 | 12 |
| 2015 | 75 | 33 | 35 | 143 |
| 2016 | 171 | 121 | 128 | 420 |
| 2017 | 159 | 156 | 154 | 469 |
| 2018 | 152 | 148 | 143 | 443 |
| 2019 | 73 | 10 | 54 | 137 |
| 2020 | 148 | 127 | 151 | 426 |

**Table 3.** Diversity statistics for microsatellites and mtDNA. N is sample size, $N_{AC}$ is average number of alleles across loci, $H_{EC}$ is Nei's gene diversity, $H_{OC}$ is observed heterozygosity, $F_{IS}$ is inbreeding co-efficient, $N_h$ is number of haplotypes, *h* is haplotype diversity, and $H_R$ is haplotype richness. Linkage disequilibrium estimates of effective size, $N_{eD}$, are also given. Genetic monitoring was not conducted in 2013. Values from 2020 monitoring year are in bold for emphasis; * indicates samples not included in diversity corrections due to smaller sample sizes. Wild caught hatchery fish (WCH) were included in genetic monitoring beginning 2014.

| | Microsatellites | | | | | | | | mtDNA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Wild-MRG** | **N** | **$N_{AC}$** | **$H_{EC}$** | **$H_{OC}$** | **$F_{IS}$** | **$N_{eD}$** | **-95** | **+95** | **N** | **$N_h$** | **h** | **$H_R$** |
| 1987 | 43 | 13.8 | 0.786 | 0.710 | 0.084 | ∞ | 139 | ∞ | 37 | 7 | 0.743 | 7.00 |
| 1999 | 46 | 12.1 | 0.800 | 0.646 | 0.196 | ∞ | ∞ | ∞ | 44 | 5 | 0.427 | 4.82 |
| 2000 | 194 | 14.1 | 0.803 | 0.698 | 0.129 | ∞ | ∞ | ∞ | 124 | 6 | 0.392 | 4.35 |
| 2001 | 128 | 14.8 | 0.796 | 0.720 | 0.097 | 2008 | 495 | ∞ | 122 | 10 | 0.643 | 7.19 |
| 2002 | 389 | 14.5 | 0.782 | 0.681 | 0.125 | 1951 | 702 | ∞ | 387 | 9 | 0.63 | 5.23 |
| 2003 | 169 | 14.7 | 0.806 | 0.710 | 0.122 | 2998 | 564 | ∞ | 167 | 9 | 0.524 | 5.89 |
| 2004 | 162 | 14.7 | 0.809 | 0.738 | 0.092 | 596 | 357 | 1559 | 161 | 11 | 0.62 | 7.47 |
| 2005 | 394 | 14.7 | 0.805 | 0.725 | 0.104 | 2724 | 1014 | ∞ | 396 | 10 | 0.612 | 6.65 |
| 2006 | 383 | 15.1 | 0.814 | 0.726 | 0.118 | 2562 | 1291 | 34064 | 378 | 10 | 0.624 | 6.67 |
| 2007 | 218 | 15.1 | 0.835 | 0.723 | 0.144 | ∞ | 1211 | ∞ | 218 | 10 | 0.579 | 6.36 |
| 2008 | 474 | 15.0 | 0.811 | 0.712 | 0.125 | 4459 | 1479 | ∞ | 466 | 11 | 0.571 | 6.36 |
| 2009 | 476 | 14.9 | 0.820 | 0.689 | 0.161 | 3608 | 1677 | ∞ | 472 | 12 | 0.59 | 6.61 |
| 2010 | 440 | 15.0 | 0.824 | 0.693 | 0.162 | ∞ | 2023 | ∞ | 433 | 9 | 0.641 | 7.01 |
| 2011 | 362 | 15.1 | 0.820 | 0.725 | 0.121 | ∞ | 3117 | ∞ | 359 | 11 | 0.634 | 6.74 |
| 2012 | 517 | 15.2 | 0.817 | 0.727 | 0.113 | 10064 | 1782 | ∞ | 522 | 10 | 0.659 | 6.71 |
| 2013 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2014 | 12 | - | - | - | - | - | - | - | - | - | - | - |
| 2015 | 144 | 15.1 | 0.805 | 0.732 | 0.092 | 468 | 281 | 1189 | 143 | 8 | 0.655 | 6.47 |
| 2016 | 420 | 15.1 | 0.812 | 0.727 | 0.106 | 1483 | 736 | ∞ | 420 | 9 | 0.75 | 7.08 |
| 2017 | 469 | 14.6 | 0.811 | 0.72 | 0.118 | 2524 | 934 | ∞ | 469 | 10 | 0.651 | 6.48 |
| 2018 | 443 | 14.8 | 0.815 | 0.72 | 0.119 | 3445 | 1048 | ∞ | 419 | 10 | 0.743 | 7.38 |
| 2019 | 134 | 14.3 | 0.808 | 0.736 | 0.092 | 1329 | 527 | ∞ | 134 | 8 | 0.677 | 6.63 |
| 2020 | 426 | 14.7 | 0.813 | 0.729 | 0.106 | 1927 | 1131 | 5627 | 426 | 10 | 0.707 | 7.28 |
| 2020 Benchmark | -- | 14.7 | 0.8 | 0.68 | -- | -- | -- | -- | -- | -- | -- | -- |

**Table 3 (cont.).** Diversity statistics for microsatellites and mtDNA. N is sample size, $N_{AC}$ is average number of alleles across loci, $H_{EC}$ is Nei's gene diversity, $H_{OC}$ is observed heterozygosity, $F_{IS}$ is inbreeding co-efficient, $N_h$ is number of haplotypes, h is haplotype diversity, and $H_R$ is haplotype richness. Linkage disequilibrium estimates of effective size, $N_{eD}$, are also given. Genetic monitoring was not conducted in 2013. Values from 2020 monitoring year are in bold for emphasis; * indicates samples not included in diversity corrections due to smaller sample sizes. Wild caught hatchery fish (WCH) were included in genetic monitoring beginning 2014.

| | Microsatellites | | | | | | | | mtDNA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Wild caught eggs** | **N** | **$N_{AC}$** | **$H_{EC}$** | **$H_{OC}$** | **$F_{IS}$** | **$N_{eD}$** | **-95** | **+95** | **N** | **$N_h$** | **h** | **$H_R$** |
| WCE_01 | 178 | 14.76 | 0.819 | 0.651 | 0.206 | 1380 | 656 | ∞ | 157 | 8 | 0.63 | 7.00 |
| WCE_SA_01 | 50 | 13.95 | 0.830 | 0.727 | 0.070 | 86 | 54 | 173 | 51 | 6 | 0.62 | 6.00 |
| WCE_AN_02 | 50 | 12.12 | 0.784 | 0.731 | 0.126 | ∞ | 238 | ∞ | 49 | 3 | 0.48 | 2.95 |
| WCE_SA_02 | 81 | 14.95 | 0.818 | 0.680 | 0.171 | ∞ | 462 | ∞ | 80 | 8 | 0.70 | 7.38 |
| WCE_SA_03 | 51 | 14.99 | 0.830 | 0.696 | 0.164 | 5009 | 308 | ∞ | 51 | 8 | 0.71 | 7.85 |
| MJO_07_005 | 54 | 15.31 | 0.827 | 0.738 | 0.091 | 60 | 48 | 79 | 53 | 7 | 0.60 | 6.73 |
| MJO_07_006 | 49 | 15.64 | 0.814 | 0.723 | 0.108 | 1065 | 196 | ∞ | 48 | 6 | 0.58 | 5.96 |
| MJO_07_015 | 49 | 15.42 | 0.818 | 0.694 | 0.154 | 871 | 270 | ∞ | 49 | 7 | 0.63 | 5.40 |
| MJO_07_016 | 50 | 15.29 | 0.837 | 0.756 | 0.097 | 2425 | 359 | ∞ | 50 | 7 | 0.60 | 5.79 |
| MJO_07_017 | 50 | 14.49 | 0.813 | 0.720 | 0.115 | 277 | 143 | 2070 | 46 | 8 | 0.76 | 6.57 |
| 2014_WCE_RG | 144 | 14.23 | 0.818 | 0.721 | 0.118 | 173 | 123 | 269 | 143 | 5 | 0.64 | 3.84 |
| 2014_WCE_RGNC | 144 | 13.54 | 0.817 | 0.721 | 0.118 | 46 | 39 | 54 | 139 | 5 | 0.58 | 3.32 |
| 2014_WCE_ALL | 288 | 14.25 | 0.821 | 0.722 | 0.122 | 117 | 88 | 162 | 281 | 5 | 0.61 | 3.68 |
| ABP14_001 | 50 | 14.40 | 0.814 | 0.706 | 0.135 | 194 | 115 | 535 | 50 | 5 | 0.41 | 3.67 |
| ABP14_002 | 49 | 15.28 | 0.838 | 0.722 | 0.140 | 189 | 114 | 485 | 48 | 5 | 0.62 | 3.72 |
| Wild-caught hatchery | | | | | | | | | | | | |
| 2014 | 184 | 14.80 | 0.831 | 0.774 | 0.069 | 133* | 101 | 184 | 182 | 6 | 0.61 | 3.87 |
| 2015 | 300 | 15.43 | 0.825 | 0.731 | 0.115 | 289 | 206 | 443 | 297 | 8 | 0.63 | 5.25 |
| 2016 | 111 | 14.23 | 0.813 | 0.706 | 0.144 | 128 | 99 | 173 | 107 | 7 | 0.69 | 4.61 |
| 2019 | 127 | 14.00 | 0.807 | 0.693 | 0.145 | 327 | 172 | 1508 | 127 | 9 | 0.68 | 5.59 |

**Table 3 (cont.).** Diversity statistics for microsatellites and mtDNA. N is sample size, $N_{AC}$ is average number of alleles across loci, $H_{EC}$ is Nei's gene diversity, $H_{OC}$ is observed heterozygosity, $F_{IS}$ is inbreeding co-efficient, $N_h$ is number of haplotypes, *h* is haplotype diversity, and $H_R$ is haplotype richness. Linkage disequilibrium estimates of effective size, $N_{eD}$, are also given. Genetic monitoring was not conducted in 2013. Values from 2020 monitoring year are in bold for emphasis; * indicates samples not included in diversity corrections due to smaller sample sizes. Wild caught hatchery fish (WCH) were included in genetic monitoring beginning 2014. Broodstock samples (Bs).

| **Captive spawned** | **N** | **$N_{AC}$** | **$H_{EC}$** | **$H_{OC}$** | **$F_{IS}$** | **$N_{eD}$** | **-95** | **+95** | **N** | **$N_h$** | ***h*** | **$H_R$** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MJO_06_29 | 50 | 11.37 | 0.804 | 0.745 | 0.074 | 42 | 29 | 69 | 50 | 5 | 0.52 | 5.00 |
| CS_01 | 64 | 12.81 | 0.794 | 0.658 | 0.172 | 44 | 36 | 55 | 58 | 5 | 0.46 | 4.98 |
| CS_AN_02 | 51 | 8.48 | 0.685 | 0.675 | 0.015 | 22 | 15 | 33 | 51 | 1 | 0 | 1.00 |
| CS_SA_02 | 53 | 13.15 | 0.802 | 0.673 | 0.163 | 73 | 53 | 111 | 53 | 6 | 0.75 | 5.92 |
| TFT_03_09 | 51 | 12.77 | 0.806 | 0.7 | 0.133 | 106 | 56 | 434 | 52 | 4 | 0.56 | 4.00 |
| CS_04 | 50 | 14.09 | 0.823 | 0.69 | 0.163 | 66 | 46 | 106 | 47 | 6 | 0.59 | 5.91 |
| TFT_04_23 | 50 | 11.65 | 0.779 | 0.683 | 0.124 | 20 | 17 | 25 | 47 | 5 | 0.59 | 5.00 |
| TFT_04_24 | 48 | 11.76 | 0.828 | 0.717 | 0.135 | 40 | 30 | 58 | 48 | 5 | 0.61 | 4.95 |
| TFT_04_25 | 50 | 11.66 | 0.81 | 0.768 | 0.053 | 25 | 20 | 32 | 53 | 6 | 0.7 | 5.93 |
| TFT_04_29 | 54 | 14.01 | 0.839 | 0.762 | 0.092 | 424 | 532 | ∞ | 53 | 5 | 0.61 | 4.90 |
| TFT_04_30 | 56 | 14.7 | 0.825 | 0.727 | 0.121 | 323 | 134 | ∞ | 45 | 5 | 0.66 | 4.79 |
| TFT_04_31 | 50 | 12.8 | 0.805 | 0.701 | 0.13 | 83 | 55 | 155 | 50 | 7 | 0.71 | 6.87 |
| TFT_05_06 | 50 | 10.31 | 0.792 | 0.649 | 0.183 | 49 | 39 | 66 | 50 | 6 | 0.63 | 5.8 |
| TFT_05_07 | 49 | 12.15 | 0.797 | 0.704 | 0.117 | 87 | 53 | 191 | 48 | 5 | 0.55 | 4.88 |
| TFT_05_08 | 50 | 11.15 | 0.804 | 0.663 | 0.178 | 32 | 27 | 40 | 49 | 5 | 0.61 | 4.93 |
| TFT_05_09 | 50 | 12.9 | 0.804 | 0.717 | 0.109 | 220 | 99 | ∞ | 50 | 4 | 0.51 | 4.00 |
| TFT_05_11 | 51 | 12.56 | 0.808 | 0.693 | 0.144 | 137 | 81 | 354 | 53 | 6 | 0.57 | 5.85 |
| MJO_06_25 | 50 | 14.85 | 0.813 | 0.721 | 0.115 | 185 | 110 | 488 | 49 | 5 | 0.64 | 4.93 |
| MJO_06_28 | 50 | 12.41 | 0.805 | 0.705 | 0.125 | 88 | 57 | 164 | 50 | 5 | 0.74 | 5.00 |

| Captive spawned | N | $N_{AC}$ | $H_{EC}$ | $H_{OC}$ | $F_{IS}$ | $N_{eD}$ | -95 | +95 | N | $N_h$ | *h* | $H_R$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MJO_07_07 | 50 | 13.16 | 0.813 | 0.739 | 0.114 | ∞ | 521 | ∞ | 50 | 5 | 0.61 | 4.87 |
| LL_11 | 50 | 14.18 | 0.829 | 0.738 | 0.11 | 302 | 123 | ∞ | 49 | 5 | 0.68 | 0.37 |
| MJO_10_05 | 49 | 14.04 | 0.839 | 0.700 | 0.167 | 260 | 87 | ∞ | 44 | 6 | 0.71 | 3.00 |
| MJO_10_06 | 49 | 12.36 | 0.782 | 0.698 | 0.108 | 59 | 32 | 163 | 49 | 6 | 0.66 | 4.88 |
| MJO_10_07 | 48 | 14.06 | 0.825 | 0.742 | 0.101 | 106 | 60 | 312 | 48 | 7 | 0.52 | 5.48 |
| MJO_11_05 | 48 | 13.97 | 0.81 | 0.73 | 0.1 | 118 | 82 | 201 | 47 | 4 | 0.59 | 3.00 |
| MJO_11_11 | 50 | 11.87 | 0.769 | 0.693 | 0.101 | 37 | 30 | 45 | 51 | 8 | 0.69 | 6.73 |
| MJO_11_12 | 50 | 11.61 | 0.785 | 0.712 | 0.094 | 27 | 21 | 35 | 50 | 5 | 0.56 | 3.92 |
| MJO_11_13 | 48 | 13.35 | 0.806 | 0.715 | 0.115 | 46 | 34 | 68 | 48 | 5 | 0.34 | 3.70 |
| MJO_11_14 | 50 | 13.77 | 0.829 | 0.754 | 0.092 | 68 | 52 | 97 | 50 | 6 | 0.47 | 4.6 |
| LL_12 | 49 | 12.48 | 0.794 | 0.684 | 0.141 | 41 | 33 | 52 | 48 | 6 | 0.63 | 4.49 |
| MJO_12_09 | 50 | 14.03 | 0.829 | 0.721 | 0.133 | 62 | 46 | 88 | 49 | 4 | 0.6 | 3.00 |
| MJO_12_10 | 50 | 14.16 | 0.81 | 0.719 | 0.113 | 121 | 69 | 371 | 50 | 7 | 0.64 | 5.71 |
| 2013_LLR | 100 | 14.51 | 0.825 | 0.765 | 0.075 | 74 | 62 | 90 | 100 | 6 | 0.63 | 4.56 |
| 2013_DEX | 100 | 14.7 | 0.818 | 0.765 | 0.066 | 112 | 87 | 152 | 99 | 6 | 0.53 | 4.23 |
| ABP13_006 | 36 | 12.22* | 0.792* | 0.716* | 0.097 | 36 | 28 | 49 | 36 | 4 | 0.67 | 7.00 |
| ABP13_002 | 50 | 12.9 | 0.799 | 0.703 | 0.122 | 27 | 22 | 33 | 50 | 3 | 0.5 | 2.00 |
| ABP14_004 | 49 | 13.9 | 0.807 | 0.683 | 0.155 | 133 | 86 | 262 | 49 | 8 | 0.56 | 6.01 |
| CSDX14_SNARRC | 150 | 14.65 | 0.827 | 0.728 | 0.12 | 179 | 127 | 279 | 147 | 7 | 0.6 | 4.49 |
| CSDX14_Los Lunas | 55 | 11.66 | 0.789 | 0.744 | 0.058 | 21 | 18 | 25 | 52 | 3 | 0.67 | 2.00 |
| ABP14-003-2011 | 50 | 14.84 | 0.808 | 0.721 | 0.12 | 771 | 196 | ∞ | 50 | 7 | 0.66 | 5.40 |
| ABP15-001 | 49 | 12.24* | 0.8 | 0.703 | 0.133 | 50 | 41 | 61 | 48 | 6 | 0.79 | 4.94 |
| ABP12-003/004 | 49 | 14.49 | 0.806 | 0.741 | 0.093 | ∞ | 271 | ∞ | 48 | 8 | 0.73 | 6.48 |

| Captive spawned | N | $N_{AC}$ | $H_{EC}$ | $H_{OC}$ | $F_{IS}$ | $N_{eD}$ | -95 | +95 | N | $N_h$ | *h* | $H_R$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15CSDX_LLSMR | 50 | 12.13* | 0.817 | 0.697 | 0.16 | 44 | 35 | 57 | 50 | 5 | 0.76 | 4.00 |
| 15CSDX | 294 | 14.38 | 0.822 | 0.735 | 0.118 | 163 | 118 | 235 | 298 | 7 | 0.74 | 4.56 |
| **Global 2016 Hatchery** | **492** | **14.73** | **0.821** | **0.726** | **0.125** | **290** | **184** | **520** | **494** | **9** | **0.748** | **5.23** |
| ABP13-003-04 WC | 50 | 13.8 | 0.808 | 0.702 | 0.134 | 407 | 190 | ∞ | 50 | 5 | 0.751 | 5.00 |
| ABP16-003 CS | 39 | 12.9 | 0.83 | 0.743 | 0.114 | 79 | 50 | 161 | 39 | 5 | 0.533 | 5.00 |
| Uvalde 2016 | 100 | 12.1 | 0.789 | 0.7 | 0.113 | 47 | 36 | 62 | 100 | 7 | 0.745 | 5.17 |
| 16CSDX-003 | 100 | 13.2 | 0.801 | 0.724 | 0.102 | 104 | 80 | 141 | 100 | 6 | 0.766 | 6.1 |
| 16CSDX-004 | 98 | 10.6 | 0.801 | 0.743 | 0.076 | 30 | 25 | 37 | 98 | 6 | 0.71 | 5.28 |
| 16CSDX-005 | 100 | 12.1 | 0.796 | 0.732 | 0.086 | 55 | 41 | 75.4 | 100 | 6 | 0.716 | 5.09 |
| **Global 2017 Hatchery** | **484** | **13.8** | **0.812** | **0.725** | **0.111** | **179** | **120** | **284** | **484** | **10** | **0.736** | **5.08** |
| ABP | 50 | 12.4 | 0.798 | 0.662 | 0.182 | 31 | 23.7 | 41 | 47 | 3 | 0.539 | 3.00 |
| 17CSDX-001 | 98 | 12.7 | 0.819 | 0.721 | 0.12 | 171 | 119 | 284 | 98 | 5 | 0.71 | 4.66 |
| 17CSDX-002 | 99 | 12.8 | 0.822 | 0.729 | 0.116 | 184 | 121 | 753 | 98 | 5 | 0.664 | 4.82 |
| 17CSDX-003 | 103 | 13 | 0.801 | 0.757 | 0.055 | 491 | 232.4 | ∞ | 102 | 6 | 0.707 | 5.41 |
| 17CSDX-004 | 99 | 13.2 | 0.812 | 0.733 | 0.097 | 211 | 134 | 432 | 96 | 6 | 0.742 | 5.54 |
| **Global 2018 Hatchery** | **449** | **13.3** | **0.815** | **0.728** | **0.109** | **297** | **214** | **441** | **441** | **6** | **0.7** | **6.00** |
| Uvalde 2016 | 100 | 12.1 | 0.789 | 0.7 | 0.113 | 47 | 36 | 62 | 100 | 7 | 0.745 | 5.17 |
| 16CSDX-003 | 100 | 13.2 | 0.801 | 0.724 | 0.102 | 104 | 80 | 141 | 100 | 6 | 0.766 | 6.1 |
| 16CSDX-004 | 98 | 10.6 | 0.801 | 0.743 | 0.076 | 30 | 24.5 | 37 | 98 | 6 | 0.71 | 5.28 |

**Table 3 (cont.).** Diversity statistics for microsatellites and mtDNA. N is sample size, $N_{AC}$ is average number of alleles across loci, $H_{EC}$ is Nei's gene diversity, $H_{OC}$ is observed heterozygosity, $F_{IS}$ is inbreeding co-efficient, $N_h$ is number of haplotypes, *h* is haplotype diversity, and $H_R$ is haplotype richness. Linkage disequilibrium estimates of effective size, $N_{eD}$, are also given. Genetic monitoring was not conducted in 2013. Values from 2020 monitoring year are in bold for emphasis. Wild caught hatchery fish (WCH) were included in genetic monitoring beginning 2014. Broodstock samples (Bs). ** year of broodstock sampling.

| Captive spawned | N | $N_{AC}$ | $H_{EC}$ | $H_{OC}$ | $F_{IS}$ | $N_{eD}$ | -95 | +95 | N | $N_h$ | *h* | $H_R$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Global 2018 Hatchery** | 449 | 13.3 | 0.815 | 0.728 | 0.109 | 297 | 214 | 441 | 441 | 6 | 0.7 | 6 |
| 18CSDX-001 | 197 | 14.2 | 0.79 | 0.701 | 0.107 | 334 | 201 | 787 | 196 | 7 | 0.711 | 4.6 |
| ABP16_001_004 | 50 | 14.4 | 0.791 | 0.682 | 0.145 | 103 | 58.7 | 288 | 50 | 6 | 0.56 | 4.7 |
| ABP18_1CS | 49 | 13.4 | 0.782 | 0.701 | 0.101 | 573 | 120 | ∞ | 47 | 7 | 0.49 | 5.74 |
| **Global 2019 Hatchery** | 296 | 14.4 | 0.804 | 0.714 | 0.115 | 422 | 286 | 732 | 291 | 8 | 0.67 | 5.9 |
| ABP19_CS | 50 | 13.92 | 0.813 | 0.701 | 0.14 | 78 | 59 | 111 | 50 | 6 | 0.635 | 6 |
| DX_YC19 | 147 | 13.40 | 0.796 | 0.615 | 0.22 | 1056 | 417 | ∞ | 147 | 6 | 0.727 | 5.52 |
| Los Lunas released | 101 | 13.87 | 0.816 | 0.737 | 0.10 | 62 | 55 | 72 | 101 | 6 | 0.704 | 5.78 |
| **Global 2020 Hatchery** | 298 | 14.58 | 0.812 | 0.676 | 0.17 | 220 | 175 | 286 | 298 | 7 | 0.716 | 5.02 |

| Broodstock | N | $N_{AC}$ | $H_{EC}$ | $H_{OC}$ | $F_{IS}$ | $N_{eD}$ | -95 | +95 | N | $N_h$ | *h* | $H_R$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABQ BioPark-Bs 2017** | 110 | 14.2 | 0.808 | 0.694 | 0.147 | 967 | 314 | ∞ | 110 | 5 | 0.659 | 4.73 |
| SNARRC- Bs 2017** | 59 | 12.7 | 0.819 | 0.692 | 0.158 | 617 | 169 | ∞ | 59 | 7 | 0.728 | 6.53 |
| ABQ BioPark- Bs 2018** | 123 | 14.9 | 0.82 | 0.729 | 0.117 | 385 | 209 | 1095 | 104 | 7 | 0.72 | 7.20 |
| SNARRC- Bs 2018** | 356 | 14.2 | 0.812 | 0.727 | 0.109 | 1551 | 370 | ∞ | 338 | 10 | 0.7 | 7.07 |
| ABQ BioPark- Bs 2019 (YC16/17) | 191 | 14.6 | 0.81 | 0.689 | 0.148 | 2892 | 569 | ∞ | 191 | 8 | 0.672 | 5.96 |
| SNARRC- Bs 2019 (YC17) | 176 | 12.9 | 0.79 | 0.7 | 0.110 | 191 | 222 | 372 | 175 | 8 | 0.705 | 5.04 |
| Los Lunas- Bs 2019 (YC18) | 186 | 14.5 | 0.81 | 0.719 | 0.118 | 449 | 222 | 3338 | 188 | 9 | 0.706 | 5.49 |
| ABQ BioPark-Bs-YC15 | 100 | 14.0 | 0.808 | 0.700 | 0.140 | 406 | 242 | 1083 | 101 | 5 | 0.499 | 4.71 |
| ABQ BioPark-Bs-YC18 | 99 | 14.4 | 0.799 | 0.719 | 0.100 | 615 | 306 | 10399 | 100 | 7 | 0.687 | 6.29 |
| SNARRC- Bs_YC17 | 99 | 15.0 | 0.827 | 0.744 | 0.100 | 160 | 124 | 217 | 99 | 8 | 0.665 | 6.68 |
| SNARRC- Bs_YC18 | 100 | 14.8 | 0.805 | 0.678 | 0.150 | 477 | 265 | 1832 | 100 | 8 | 0.709 | 7.37 |
| SNARRC- Bs_YC19 | 96 | 15.5 | 0.821 | 0.738 | 0.100 | 495 | 239 | 2047 | 97 | 9 | 0.649 | 7.13 |
| Los Lunas-Bs- ABP18-4WI | 138 | 15.6 | 0.833 | 0.731 | 0.112 | ∞ | 2285 | ∞ | 137 | 10 | 0.740 | 7.48 |

**Table 4.** MtDNA haplotype frequencies (provided as percentage of sample size) for the middle Rio Grande population, wild caught hatchery fish, fish reared from wild-caught eggs, and fish reared from captive spawning. Values from 2020 monitoring year are shaded for emphasis.

| | A | C | D | E | F | I | J | K | M | O | P | Q | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1987** | 45.95 | 16.22 | 16.22 | 5.41 | 8.11 | - | - | 2.70 | 5.41 | - | - | - | - | - | - |
| **1999** | 75.00 | - | 11.36 | 6.82 | 4.55 | - | - | 2.27 | - | - | - | - | - | - | - |
| **2000** | 76.98 | 0.79 | 4.76 | 4.76 | 11.90 | - | - | 0.79 | - | - | - | - | - | - | - |
| **2001** | 57.43 | 10.89 | 4.95 | 3.96 | 9.90 | 0.99 | 0.99 | 8.91 | 0.99 | 0.99 | - | - | - | - | - |
| **2002** | 55.56 | 19.90 | 13.70 | 1.03 | 5.94 | - | 0.26 | 3.36 | - | 0.26 | - | - | - | - | - |
| **2003** | 67.07 | 5.39 | 14.97 | 2.99 | 5.39 | - | 0.60 | 1.20 | 0.60 | 1.80 | - | - | - | - | - |
| **2004** | 59.63 | 8.70 | 10.56 | 1.86 | 7.45 | 1.24 | - | 4.97 | 1.86 | 3.11 | 0.62 | - | - | - | - |
| **2005** | 59.69 | 12.76 | 8.93 | 2.81 | 8.42 | 1.53 | 0.26 | 1.79 | 2.81 | 1.02 | - | - | - | - | - |
| **2006** | 58.58 | 13.72 | 9.23 | 4.75 | 4.75 | 0.26 | - | 4.75 | 2.90 | 0.79 | - | - | - | 0.26 | - |
| **2007** | 62.84 | 11.01 | 8.26 | 2.29 | 8.72 | 0.46 | - | 3.67 | 0.46 | 1.83 | - | 0.46 | - | - | - |
| **2008** | 63.46 | 11.97 | 7.91 | 2.56 | 6.62 | 0.64 | - | 4.49 | 0.85 | 0.64 | 0.21 | - | 0.64 | - | - |
| **2009** | 61.57 | 14.01 | 7.64 | 2.76 | 6.37 | 0.64 | 0.42 | 3.40 | 1.70 | 1.06 | 0.21 | - | 0.21 | - | - |
| **2010** | 57.11 | 12.09 | 9.72 | 3.08 | 7.11 | 1.42 | - | 5.45 | 1.66 | 2.37 | - | - | - | - | - |
| **2011** | 57.38 | 14.21 | 10.86 | 2.79 | 6.41 | 0.56 | - | 3.06 | 3.06 | 1.11 | - | 0.28 | 0.28 | - | - |
| **2012** | 54.28 | 17.32 | 9.53 | 3.70 | 7.59 | 0.39 | 0.39 | 2.92 | 2.14 | 1.75 | - | - | - | - | - |
| **2015** | 55.24 | 12.59 | 12.59 | 1.40 | 9.79 | - | - | 2.10 | 2.10 | 4.20 | - | - | - | - | - |
| **2016** | 40.51 | 25.32 | 9.37 | 1.77 | 7.09 | 0.76 | - | 3.04 | 3.29 | 8.86 | - | - | - | - | - |
| **2017** | 55.44 | 14.93 | 10.66 | 1.28 | 7.04 | 0.21 | - | 1.92 | 2.13 | 6.18 | - | - | - | - | 0.21 |
| **2018** | 44.74 | 16.51 | 11.72 | 1.44 | 9.09 | 0.72 | - | 4.07 | 2.87 | 7.89 | - | - | - | - | 0.96 |
| **2019** | 50.76 | 23.48 | 3.79 | 3.03 | 4.55 | - | - | 3.03 | 1.52 | 9.85 | - | - | - | - | - |
| **2020** | 48.70 | 19.62 | 8.27 | 1.42 | 7.33 | 1.65 | - | 3.55 | 1.65 | 6.86 | - | - | - | - | 0.95 |

**Table 4 (cont.).** MtDNA haplotype frequencies for the wild middle Rio Grande population, wild caught hatchery fish, fish reared from wild-caught eggs, and fish reared from captive spawning. Values from 2020 monitoring year are shaded for emphasis.

| **Wild caught eggs** | **A** | **C** | **D** | **E** | **F** | **I** | **J** | **K** | **M** | **N** | **O** | **P** | **Q** | **S** | **T** | **V** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WCE_01* | 57.3 | 19.7 | 5.1 | 6.4 | 6.4 | - | - | 3.2 | 1.3 | 0.6 | - | - | - | - | - | - |
| WCE_SA_01 | 56.9 | 13.7 | 5.9 | 5.9 | 9.8 | - | - | 7.8 | - | - | - | - | - | - | - | - |
| WCE_AN_02 | 65.3 | 2.0 | 32.7 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| WCE_SA_02 | 48.8 | 22.5 | 5.0 | 1.3 | 13.8 | - | - | 5.0 | 3.8 | - | - | - | - | - | - | - |
| WCE_SA_03 | 49.0 | 7.8 | 19.6 | 5.9 | 9.8 | - | - | 3.9 | 2.0 | - | 2.0 | - | - | - | - | - |
| MJO_07_005 | 60.4 | 9.4 | 1.9 | 1.9 | 17.0 | - | 1.9 | 7.5 | - | - | - | - | - | - | - | - |
| MJO_07_006 | 60.4 | 8.3 | 12.5 | 2.1 | 8.3 | - | - | 4.2 | - | - | 4.2 | - | - | - | - | - |
| MJO_07_015 | 57.1 | 22.4 | 4.1 | 2.0 | 4.1 | - | - | 8.2 | 2.0 | - | - | - | - | - | - | - |
| MJO_07_016 | 62.0 | 12.0 | 6.0 | - | 8.0 | - | - | 4.0 | 4.0 | - | 4.0 | - | - | - | - | - |
| MJO_07_017 | 43.5 | 19.6 | 6.5 | 4.3 | 13.0 | - | - | 8.7 | 2.2 | - | - | - | 2.2 | - | - | - |
| 2014_WCE_RG | 54.2 | 7.0 | 22.5 | - | 4.9 | - | - | - | - | - | 11.3 | - | - | - | - | - |
| 2014_WCE_RGNC | 58.3 | 7.2 | 28.1 | - | 1.4 | - | - | - | - | - | 5.0 | - | - | - | - | - |
| 2014_WCE_ALL | 56.2 | 7.1 | 25.3 | - | 3.2 | - | - | - | - | - | 8.2 | - | - | - | - | - |
| ABP14-001 | 76.0 | 8.0 | 2.0 | 4.0 | 10.0 | - | - | - | - | - | - | - | - | - | - | - |
| ABP14-002 | 56.0 | 4.0 | 19.0 | - | 2.0 | - | - | - | - | - | 19.0 | - | - | - | - | - |
| **Captive spawned** | | | | | | | | | | | | | | | | |
| MJO_06_29 | 68.0 | 14.0 | 8.0 | - | 6.0 | - | - | - | 4.0 | - | - | - | - | - | - | - |
| CS_01 | 72.4 | 5.2 | - | 3.4 | 6.9 | - | - | 12.1 | - | - | - | - | - | - | - | - |
| CS_AN_02 | - | - | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CS_SA_02 | 43.4 | 7.5 | 17.0 | 13.2 | 17.0 | - | - | - | - | - | - | 1.9 | - | - | - | - |
| TFT_03_09 | 59.6 | 26.9 | 3.8 | - | - | - | - | 9.6 | - | - | - | - | - | - | - | - |
| CS_04 | 59.6 | 25.5 | 2.1 | - | 4.3 | - | - | 6.4 | - | - | 2.1 | - | - | - | - | - |
| TFT_04_23 | 61.7 | 4.3 | 19.1 | - | - | - | - | 4.3 | - | - | 10.6 | - | - | - | - | - |
| TFT_04_24 | 58.3 | 12.5 | 20.8 | - | 2.1 | - | - | 6.3 | - | - | - | - | - | - | - | - |
| TFT_04_25 | 43.4 | 5.7 | 11.3 | 5.7 | 28.3 | - | - | 5.7 | - | - | - | - | - | - | - | - |
| TFT_04_29 | 56.6 | 24.5 | - | 7.5 | - | - | - | 9.4 | 1.9 | - | - | - | - | - | - | - |

**Table 4 (cont.).** MtDNA haplotype frequencies for the middle Rio Grande population, wild caught hatchery fish, fish reared from wild-caught eggs, and fish reared from captive spawning. Values from 2020 monitoring year are shaded for emphasis.

| Captive spawned | A | C | D | E | F | I | J | K | M | N | O | P | Q | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TFT04_30 | 40.0 | 33.3 | - | - | - | - | - | 24.4 | - | - | - | 2.2 | - | - | - | - |
| TFT_04_31 | 42.0 | 34.0 | 2.0 | - | 6.0 | - | - | 4.0 | 10.0 | - | 2.0 | - | - | - | - | - |
| TFT_05_06 | 50.0 | 36.0 | 2.0 | - | 2.0 | - | - | 8.0 | 2.0 | - | - | - | - | - | - | - |
| TFT_05_07 | 62.5 | 29.2 | 2.1 | 6.3 | - | - | - | - | - | - | - | - | - | - | - | - |
| TFT_05_08 | 59.2 | 8.2 | - | 10.2 | - | - | - | 22.4 | - | - | - | - | - | - | - | - |
| TFT_05_09 | 68.0 | 16 | - | - | - | - | - | 12 | 4.0 | - | - | - | - | - | - | - |
| TFT_05_11 | 62.3 | 5.7 | 11.3 | 1.9 | 17.0 | - | - | - | 1.9 | - | - | - | - | - | - | - |
| MJO_06_25 | 55.1 | 24.5 | 6.1 | - | 6.1 | - | - | 8.2 | - | - | - | - | - | - | - | - |
| MJO_06_28 | 40.0 | 14.0 | 22.0 | - | 22.0 | - | - | 2.0 | - | - | - | - | - | - | - | - |
| MJO_07_07 | 56.0 | 2.0 | 12.0 | 28.0 | 2.0 | - | - | - | - | - | - | - | - | - | - | - |
| LL_11 | 46.9 | 22.4 | 24.5 | - | 4.1 | - | - | - | 2.0 | - | - | - | - | - | - | - |
| MJO_10_05 | 47.7 | 18.2 | 15.9 | - | 13.6 | - | - | 2.3 | - | - | 2.3 | - | - | - | - | - |
| MJO_10_06 | 53.1 | 22.4 | 4.1 | 6.1 | - | 10.2 | - | - | 4.1 | - | - | - | - | - | - | - |
| MJO_10_07 | 68.8 | 6.3 | 4.2 | 2.1 | 8.3 | - | - | 8.3 | - | - | - | - | 2.1 | - | - | - |
| MJO_11_05 | 59.6 | 21.3 | 10.6 | - | 8.5 | - | - | - | - | - | - | - | - | - | - | - |
| MJO_11_11 | 52.9 | 5.9 | 3.9 | 3.9 | 3.9 | - | - | 17.6 | 5.9 | - | - | - | - | - | - | - |
| MJO_11_12 | 64.0 | 12 | 4.0 | - | - | - | - | - | 14 | - | 6.0 | - | - | - | - | - |
| MJO_11_13 | 81.3 | 6.3 | 6.3 | 4.2 | - | - | - | - | 2.1 | - | - | - | - | - | - | - |
| MJO_11_14 | 72.0 | 4.0 | 6.0 | 4.0 | - | - | - | 12 | - | - | 2.0 | - | - | - | - | - |
| LL_12 | 56.3 | 4.2 | 12.5 | - | 22.9 | - | - | 2.1 | - | - | - | - | 2.1 | - | - | - |
| MJO_12_09 | 59.2 | 18.4 | 8.2 | - | 14.3 | - | - | - | - | - | - | - | - | - | - | - |
| MJO_12_10 | 58.0 | 8.0 | 10.0 | - | 10.0 | - | - | 6.0 | - | - | 2.0 | - | 6.0 | - | - | - |
| 2013_LLR | 57.0 | 20.0 | 4.0 | 9.0 | 3.0 | - | - | - | - | - | 7.0 | - | - | - | - | - |
| 2013_DEX | 66.7 | 11.1 | 9.1 | - | 5.1 | - | - | - | - | - | 7.1 | - | - | - | - | 1.0 |
| ABP13_002 | 66.0 | 8.0 | 26.0 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ABP13_006 | 42.0 | 14.0 | 39.0 | - | - | - | - | - | - | - | 6.0 | - | - | - | - | - |

**Table 4 (cont.).** MtDNA haplotype frequencies for the wild middle Rio Grande population, wild caught hatchery fish, fish reared from wild-caught eggs, and fish reared from captive spawning. Values from 2020 monitoring year are shaded for emphasis.

| Captive spawned | A | C | D | E | F | I | J | K | M | N | O | P | Q | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABP14_004 | 65.0 | 6.0 | 2.0 | 2.0 | 12.0 | - | - | 8.0 | 2.0 | - | 2.0 | - | - | - | - | - |
| CSDX14_SNARRC | 61.0 | 13.0 | 0.14 | - | 1.0 | - | - | 3.0 | 2.0 | - | 7.0 | - | - | - | - | - |
| CSDX14_LL | 37.0 | - | 38.0 | - | - | - | - | 25.0 | - | - | - | - | - | - | - | - |
| 15CSDX | 41.6 | 18.1 | 7.4 | - | 12.4 | - | - | 1.7 | 0.3 | - | 18.5 | - | - | - | - | - |
| ABP12_03_04 | 46.9 | 16.3 | 4.1 | 2.0 | 8.2 | 2.0 | - | - | 6.1 | - | 12.2 | - | 2.0 | - | - | - |
| ABP14_03_2011 | 54.0 | 18.0 | 14.0 | 4.0 | 6.0 | - | - | 2 | - | - | 2.0 | - | - | - | - | - |
| ABP15_01 | 22.4 | 30.6 | 6.1 | - | 12.2 | - | - | - | - | - | 22.4 | - | - | - | - | - |
| LLSMR | 40.0 | 18.0 | 20 | - | - | - | - | 12 | - | - | 10.0 | - | - | - | - | - |
| ABP13-003-04 WC | 40.0 | 24.0 | 12 | - | 16 | - | - | - | - | - | 8.0 | - | - | - | - | - |
| ABP16-003 CS | 64.0 | 25.6 | 2.6 | 5.1 | - | - | - | - | 2.6 | - | - | - | - | - | - | - |
| Uvalde 2016 | 38.0 | 21.0 | 23 | - | - | - | - | - | - | - | 14.0 | - | - | - | - | - |
| 16CSDX-003 | 37 | 22.2 | 14.1 | 3.0 | 17.1 | - | - | 1.0 | - | - | 5.1 | - | - | - | - | - |
| 16CSDX-004 | 37.8 | 35.7 | 5.1 | - | - | - | - | 6.1 | - | - | 14.3 | - | - | 1.0 | - | - |
| 16CSDX-005 | 39.0 | 28.0 | 24 | - | 5.0 | 3.0 | - | - | - | - | 1.0 | - | - | - | - | - |
| ABP18 | 55.3 | 40.4 | 4.3 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17CSDX_001 | 40.8 | 25.5 | 24.5 | - | 2.0 | - | - | - | - | - | 7.1 | - | - | - | - | - |
| 17CSDX_002 | 52.0 | 17.3 | 17.2 | - | 10.2 | - | - | - | - | - | 3.1 | - | - | - | - | - |
| 17CSDX_003 | 41.2 | 18.6 | 29.1 | 2.0 | 2.9 | - | - | - | - | - | 5.9 | - | - | - | - | - |
| 17CSDX_004 | 39.6 | 20.8 | 20.2 | 1.0 | 12.5 | - | - | - | - | - | 5.2 | - | - | - | - | - |
| 18CSDX_001 | 44.9 | 20.4 | 19.9 | 1.5 | 4.1 | - | - | 0.5 | - | - | 8.7 | - | - | - | - | - |
| ABP18_CS | 67.3 | 10.2 | 0.2 | 0.2 | 0.2 | - | - | - | 4.1 | - | 8.0 | - | - | - | - | - |
| ABP16_001_004 | 60.0 | 24 | 2.0 | - | 8.0 | - | - | - | 4.0 | | 2.0 | - | - | - | - | - |
| ABP19_CS | 57.1 | 18.4 | 6.1 | - | 2.0 | - | - | 8.2 | - | - | 8.2 | - | - | - | - | - |
| DX_YC19 | 43.2 | 15.8 | 17.8 | - | 2.7 | - | - | - | 2.1 | - | 18.5 | - | - | - | - | - |
| Los Lunas released | 42.0 | 14.0 | 32.0 | - | 4.0 | - | - | - | 3.0 | - | 5.0 | - | - | - | - | - |

**Table 5.** MtDNA haplotype frequencies for broodstock held at ABQ BioPark, Southwestern ARRC and the Los Lunas SMR. Samples collected for the 2020 monitoring year are shaded for emphasis.

| Broodstock | A | C | D | E | F | I | J | K | M | N | O | P | Q | S | T | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ABQ Biopark-Bs-2017** | 50.0 | 26.3 | 10.9 | - | 2.7 | - | - | - | - | - | 9.1 | - | - | - | - | - |
| **SNARRC- Bs-2017** | 44.1 | 22 | 15.2 | 3.4 | 5.1 | - | - | - | - | - | 6.8 | - | - | - | - | - |
| **ABQ Biopark-Bs-2018** | 47.1 | 26 | 6.7 | 0.96 | 6.7 | - | - | - | 3.9 | - | 8.7 | - | - | - | - | - |
| **SNARRC- Bs-2018** | 49.4 | 17.1 | 13.6 | 1.2 | 6.8 | 1.5 | - | 0.9 | 0.6 | - | 8.3 | - | - | - | - | 0.6 |
| **ABQ Biopark-Bs-2019** | 53 | 14.2 | 15.3 | - | 4.9 | 0.6 | - | 3.3 | 3.3 | - | 5.5 | - | - | - | - | - |
| **SNARRC- Bs-2019** | 47.6 | 17.1 | 18.2 | 1.1 | 4.8 | - | - | 2.7 | 0.5 | - | 7.5 | - | - | - | - | 0.5 |
| **Los Lunas- Bs-2019** | 47.9 | 14.8 | 13.6 | - | 3.6 | 0.6 | - | 2.4 | 0.6 | - | 16.6 | - | - | - | - | - |
| | | | | | | | | | | | | | | | | |
| **ABQ Biopark-Bs-2015** | 68.1 | 19.2 | 6.4 | - | 2.1 | - | - | - | - | - | 4.3 | - | - | - | - | - |
| **ABQ Biopark-Bs-2018** | 43.4 | 34.3 | 8.1 | 3.0 | 6.1 | - | - | - | 1.0 | - | 4.0 | - | - | - | - | - |
| **SNARRC- Bs-YC2017** | 53.1 | 18.4 | 14.3 | 2.0 | 4.1 | 1.0 | - | 1.0 | - | - | 6.1 | - | - | - | - | - |
| **SNARRC- Bs-YC2018** | 48.0 | 22.5 | 8.2 | 1.0 | 6.1 | 5.1 | - | 5.1 | 4.1 | - | - | - | - | - | - | - |
| **SNARRC- Bs-YC2019** | 54.2 | 21.9 | 4.2 | 1.0 | 11.5 | 1.0 | - | 3.1 | 1.0 | - | 2.1 | - | - | - | - | - |
| **Los Lunas-Bs- ABP18-4WI** | 41.9 | 25.7 | 12.5 | 0.7 | 9.6 | 0.7 | - | 2.2 | 2.2 | - | 4.4 | - | - | - | - | 0.7 |

**Table 6.** Pairwise $F_{ST}$ values (below diagonal) and associated p-values (above diagonal) calculated based on microsatellites for 'wild' (2019 and 2020), captive stocks released in fall 2019 and spring 2020, and refugial broodstocks (BS) sampled in fall 2019 and spring 2020. Significant values following Bonferroni correction are indicated with an asterisk. SNARRC/DX- Southwestern ARRC.

| | Wild 2019 | Wild 2020 | ABQ BioPark Bs YC15 | ABQ BioPark Bs YC18 | ABP19-1CS | Los Lunas-released | DX_YC19 | SNARRC Bs YC17 | SNARRC Bs YC18 | SNARRC Bs YC19 | Los Lunas-Bs-ABP18-4WI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wild 2019 | * | 0.0313 | **0.00001** | 0.0010 | 0.0967 | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| Wild 2020 | 0.001 | * | **0.00001** | **0.00001** | 0.0088 | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| ABQ BioPark Bs YC15 | **0.006** | **0.004** | * | 0.0029 | 0.0107 | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| ABQ BioPark Bs YC18 | 0.004 | **0.003** | 0.005 | * | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| ABP19-1CS | 0.003 | 0.004 | 0.005 | **0.009** | * | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| Los Lunas-released | **0.010** | **0.009** | **0.014** | **0.012** | **0.016** | * | **0.00001** | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| DX_YC19 | **0.023** | **0.025** | **0.028** | **0.028** | **0.026** | **0.035** | * | **0.00001** | **0.00001** | **0.00001** | **0.00001** |
| SNARRC Bs_YC17 | **0.014** | **0.012** | **0.016** | **0.013** | **0.014** | **0.020** | **0.046** | * | 0.001 | 0.004 | 0.001 |
| SNARRC Bs_YC18 | **0.015** | **0.014** | **0.016** | **0.015** | **0.013** | **0.021** | **0.038** | 0.005 | * | 0.033 | **0.00001** |
| SNARRC-Bs_YC19 | **0.011** | **0.008** | **0.011** | **0.010** | **0.010** | **0.016** | **0.032** | 0.004 | 0.003 | * | 0.002 |
| Los Lunas-Bs-ABP18-4WI | **0.015** | **0.011** | **0.017** | **0.013** | **0.015** | **0.019** | **0.042** | 0.004 | **0.007** | 0.004 | * |

**Table 7.** Pairwise $\Phi_{ST}$ values (below diagonal) and associated p-values (above diagonal) calculated based on mtDNA haplotype frequencies for 'wild', captive stocks, and broodstock collections (BS) sampled in 2019-2020. Bold text denotes significant values following Bonferroni correction. SNARRC- Southwestern ARRC.

| | Wild 2019 | Wild 2020 | ABQ BioPark Bs YC15 | ABQ BioPark Bs YC18 | SNARRC Bs YC17 | SNARRC Bs YC18 | SNARRC Bs YC19 | Los Lunas-Bs- ABP18-4WI | ABP19-1CS | Los Lunas-released | DX_YC19 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wild 2019 | * | 0.613 | 0.121 | 0.377 | 0.281 | 0.344 | 0.05 | 0.316 | 0.334 | 0.001 | **0.0001** |
| Wild 2020 | -0.002 | * | 0.114 | 0.095 | 0.316 | 0.52 | 0.074 | 0.205 | 0.363 | **0.0001** | **0.0001** |
| ABQ BioPark Bs YC15 | 0.009 | 0.006 | * | 0.016 | 0.063 | 0.429 | 0.27 | 0.016 | 0.521 | **0.0001** | **0.0001** |
| ABQ BioPark Bs YC18 | -0.0005 | 0.007 | 0.03 | * | 0.193 | 0.088 | 0.009 | 0.586 | 0.061 | 0.007 | **0.0001** |
| SNARRC Bs YC17 | 0.002 | 0.001 | 0.016 | 0.005 | * | 0.159 | 0.02 | 0.313 | 0.192 | 0.015 | 0.004 |
| SNARRC Bs YC18 | 0.0003 | -0.002 | -0.002 | 0.012 | 0.006 | * | 0.541 | 0.132 | 0.675 | **0.0001** | **0.0001** |
| SNARRC Bs YC19 | 0.015 | 0.008 | 0.003 | 0.033 | 0.027 | -0.003 | * | 0.018 | 0.259 | **0.0001** | **0.0001** |
| Los Lunas-Bs-ABP18-4WI | 0.001 | 0.002 | 0.027 | -0.003 | 0.001 | 0.008 | 0.025 | * | 0.074 | 0.008 | 0.001 |
| ABP19-1CS | 0.001 | -0.0002 | -0.005 | 0.021 | 0.007 | -0.007 | 0.004 | 0.018 | * | 0.002 | **0.0001** |
| Los Lunas-released | 0.044 | 0.051 | 0.096 | 0.033 | 0.027 | 0.075 | **0.11** | 0.028 | 0.074 | * | 0.137 |
| DX_YC19 | **0.086** | **0.087** | **0.141** | **0.066** | 0.05 | **0.107** | **0.15** | 0.053 | **0.113** | 0.008 | * |

**Table 8.** Pairwise $F_{ST}$ values (below diagonal) calculated based on microsatellite data for samples collected from the middle Rio Grande between 1987 and 2020. Following Bonferroni correction, no values were significantly different from zero.

| | **1987** | **1999** | **2000** | **2001** | **2002** | **2003** | **2004** | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** | **2011** | **2012** | **2015** | **2016** | **2017** | **2018** | **2019** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1987** | * | | | | | | | | | | | | | | | | | | | |
| **1999** | 0.006 | * | | | | | | | | | | | | | | | | | | |
| **2000** | 0.002 | 0.004 | * | | | | | | | | | | | | | | | | | |
| **2001** | 0.001 | 0.007 | 0.002 | * | | | | | | | | | | | | | | | | |
| **2002** | 0.003 | 0.007 | 0.004 | 0.004 | * | | | | | | | | | | | | | | | |
| **2003** | 0.000 | 0.005 | 0.002 | 0.001 | 0.003 | * | | | | | | | | | | | | | | |
| **2004** | 0.001 | 0.007 | 0.002 | 0.001 | 0.004 | 0.000 | * | | | | | | | | | | | | | |
| **2005** | 0.001 | 0.005 | 0.001 | 0.002 | 0.004 | 0.000 | 0.002 | * | | | | | | | | | | | | |
| **2006** | 0.004 | 0.005 | 0.003 | 0.002 | 0.006 | 0.001 | 0.001 | 0.002 | * | | | | | | | | | | | |
| **2007** | 0.008 | 0.011 | 0.010 | 0.010 | 0.017 | 0.009 | 0.008 | 0.010 | 0.007 | * | | | | | | | | | | |
| **2008** | 0.001 | 0.006 | 0.003 | 0.004 | 0.008 | 0.002 | 0.004 | 0.003 | 0.003 | 0.007 | * | | | | | | | | | |
| **2009** | 0.002 | 0.006 | 0.003 | 0.005 | 0.007 | 0.003 | 0.003 | 0.003 | 0.003 | 0.005 | 0.001 | * | | | | | | | | |
| **2010** | 0.004 | 0.005 | 0.005 | 0.005 | 0.009 | 0.003 | 0.002 | 0.004 | 0.003 | 0.005 | 0.002 | 0.001 | * | | | | | | | |
| **2011** | 0.002 | 0.005 | 0.005 | 0.005 | 0.008 | 0.003 | 0.003 | 0.004 | 0.003 | 0.006 | 0.001 | 0.001 | 0.001 | * | | | | | | |
| **2012** | 0.003 | 0.005 | 0.004 | 0.004 | 0.007 | 0.002 | 0.003 | 0.003 | 0.002 | 0.005 | 0.002 | 0.001 | 0.001 | 0.000 | * | | | | | |
| **2015** | 0.005 | 0.008 | 0.006 | 0.005 | 0.008 | 0.006 | 0.007 | 0.006 | 0.006 | 0.012 | 0.005 | 0.005 | 0.006 | 0.004 | 0.004 | * | | | | |
| **2016** | 0.006 | 0.007 | 0.007 | 0.006 | 0.009 | 0.006 | 0.006 | 0.006 | 0.005 | 0.010 | 0.005 | 0.005 | 0.004 | 0.003 | 0.004 | 0.000 | * | | | |
| **2017** | 0.008 | 0.008 | 0.008 | 0.006 | 0.010 | 0.007 | 0.007 | 0.006 | 0.006 | 0.010 | 0.006 | 0.006 | 0.005 | 0.004 | 0.005 | 0.000 | 0.000 | * | | |
| **2018** | 0.008 | 0.008 | 0.007 | 0.007 | 0.012 | 0.007 | 0.008 | 0.006 | 0.006 | 0.011 | 0.005 | 0.006 | 0.006 | 0.005 | 0.005 | 0.001 | 0.000 | 0.002 | * | |
| **2019** | 0.008 | 0.010 | 0.008 | 0.006 | 0.013 | 0.007 | 0.008 | 0.007 | 0.007 | 0.011 | 0.006 | 0.006 | 0.006 | 0.005 | 0.006 | 0.001 | 0.001 | 0.001 | 0.002 | * |
| **2020** | 0.008 | 0.007 | 0.008 | 0.007 | 0.011 | 0.007 | 0.008 | 0.006 | 0.006 | 0.011 | 0.005 | 0.006 | 0.006 | 0.004 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 |

**Figure 1.** Annual diversity metrics calculated from microsatellite loci. Estimates of gene diversity and heterozygosity obtained from resampling of microsatellites ($H_{EC}$ and $H_{OC}$) and haplotype diversity (*h*) from mitochondrial data are shown in the upper panel, and number of alleles ($N_{AC}$) and haplotypes ($H_R$) are shown in the lower panel. Dashed ($H_{EC}$ and $N_{AC}$) and dotted ($H_{OC}$) lines indicate diversity benchmarks obtained using a resampling procedure and correspond to a minimum sample size of n=43.

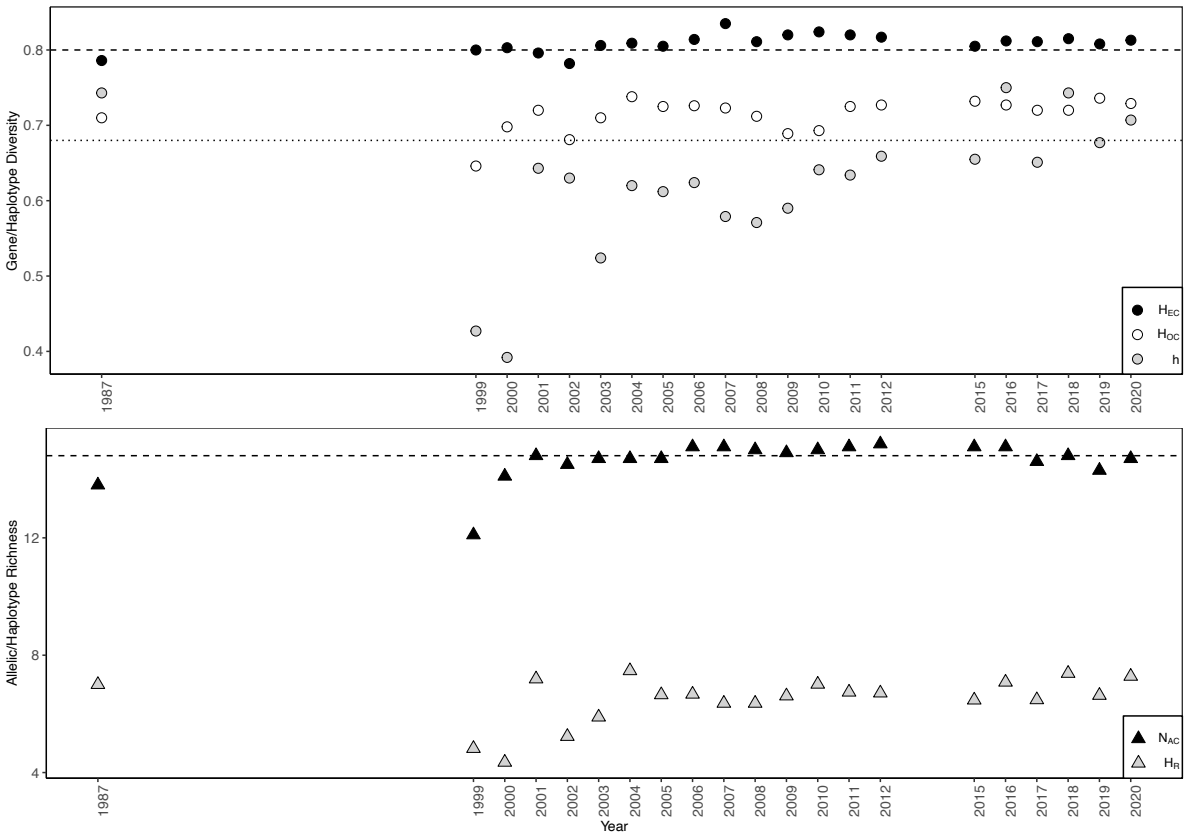

**Figure 2.** Annual diversity metrics of wild Rio Grande Silvery Minnow by reach. Microsatellites diversity estimates, $H_{EC}$ (top), $H_{OC}$ (middle), $N_{AC}$ (bottom) were corrected for differences in sample sizes across years by resampling.

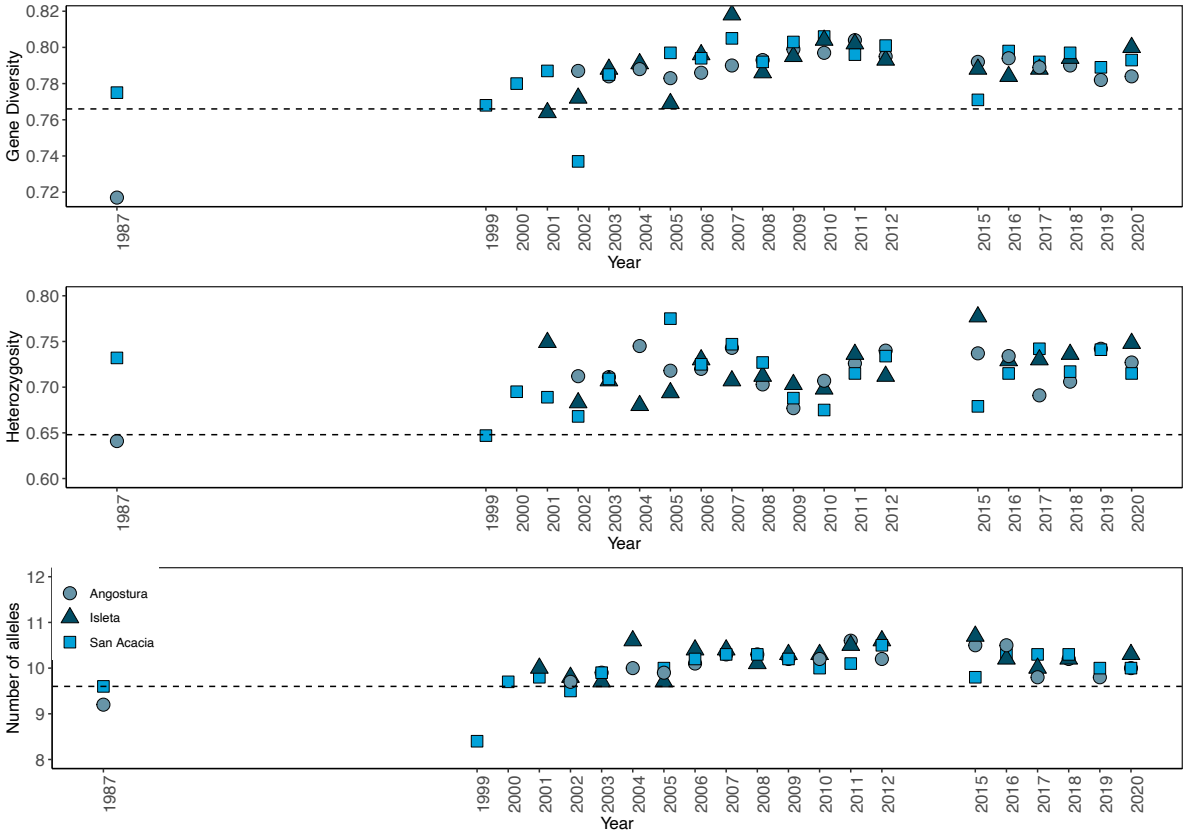

**Figure 3.** Annual mtDNA diversity metrics of wild Rio Grande Silvery Minnow by year and river reach. Estimates of mtDNA haplotype diversity are shown in the upper panel and haplotype richness are shown in the lower panel.

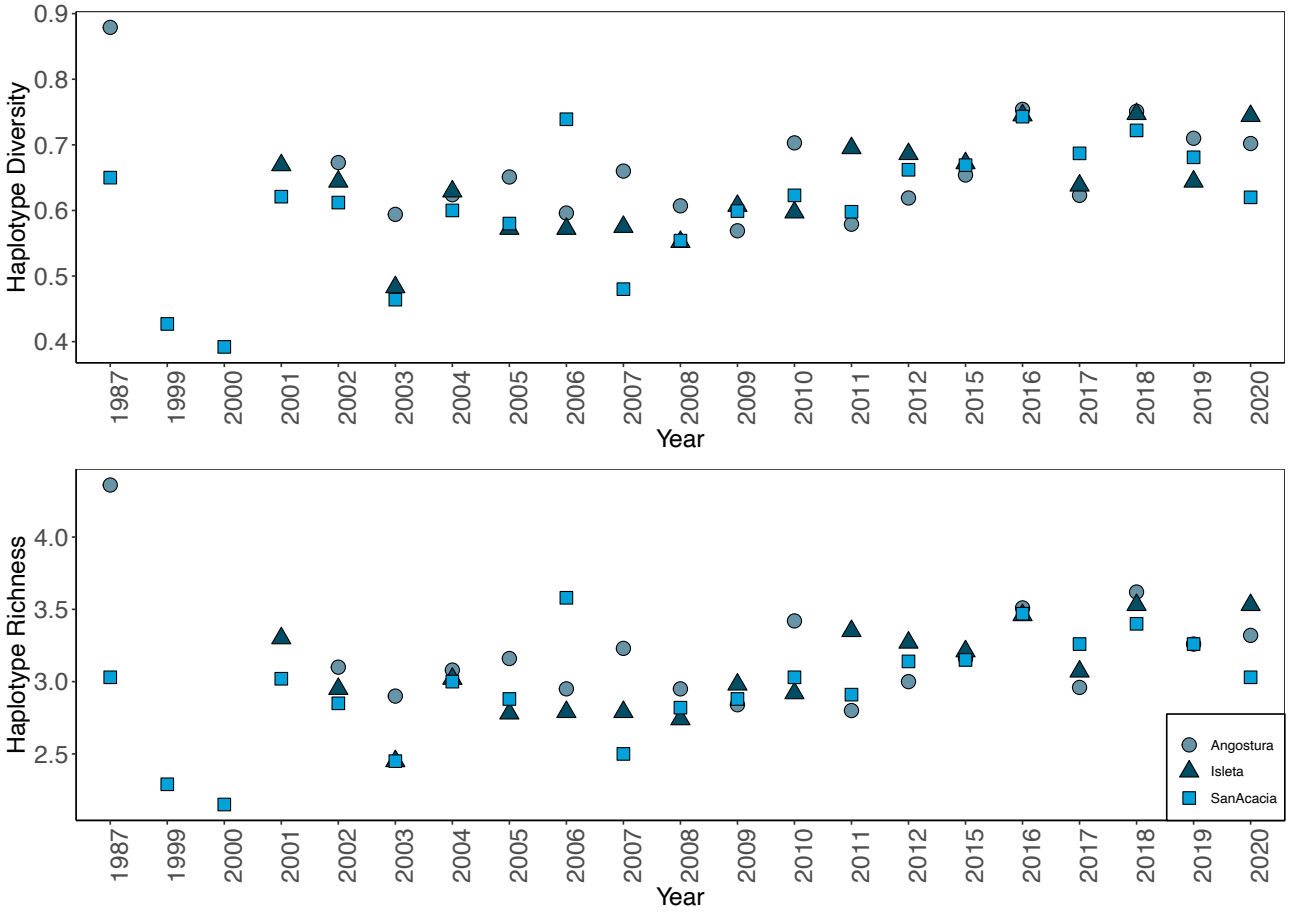

**Figure 4.** Variance effective size ($N_{eV}$) calculated from microsatellite data, as based on MLNE (upper), temporal (middle), and TEMPOFS (lower) estimates and associated 95% CIs. Mean TEMPOFS estimate from 2011-2012 (value not shown) was infinite, and upper error bars extending to y-maxima indicate infinite upper bounded 95% CI.

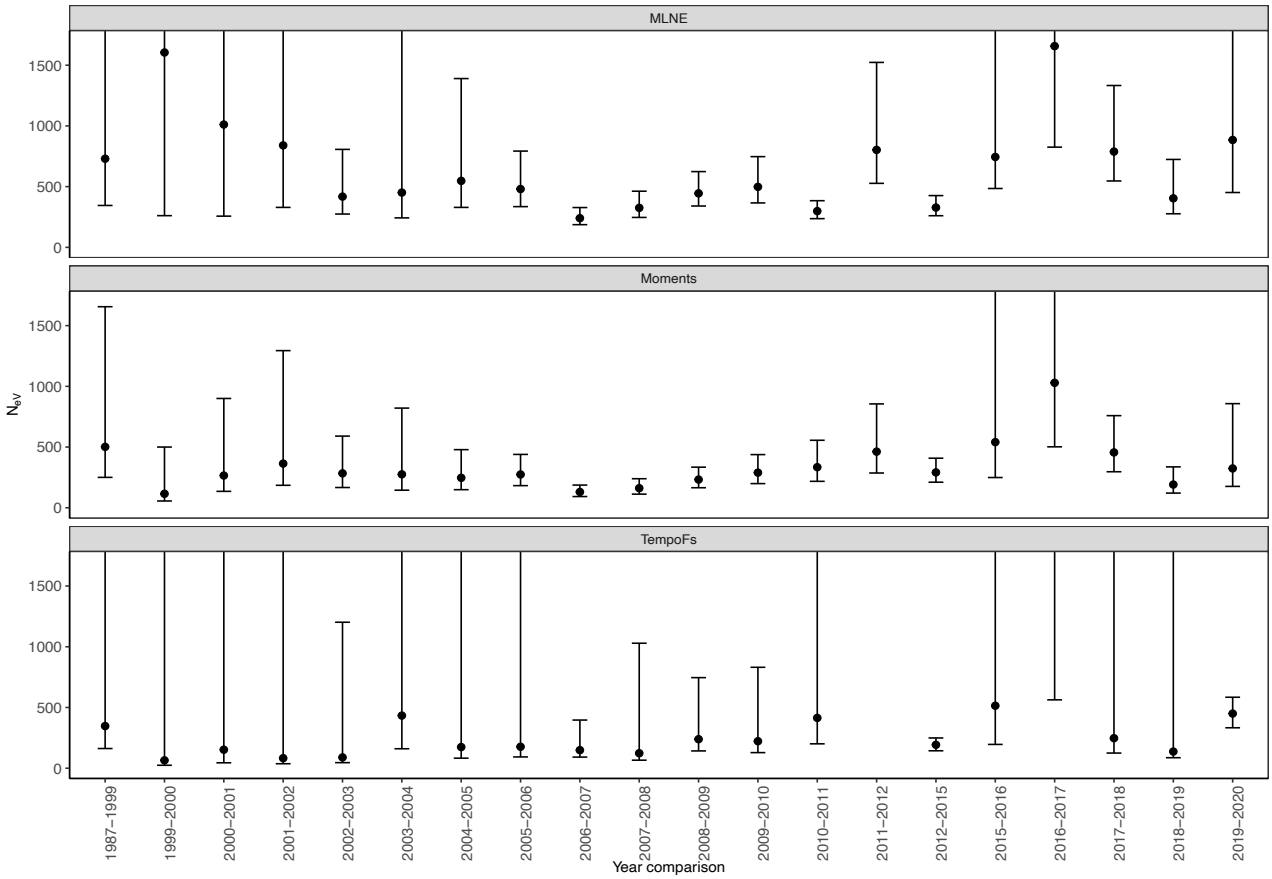

**Figure 5.** Female variance effective size estimates ($N_{ef}$) and their associated 95% CIs, based on mtDNA data and calculated using MLNE (upper) and temporal (moments) (lower) methods. Infinite mean estimates are indicated by points falling outside of the plot area and upper error bars extending to y-maxima indicate infinite upper bounded 95% CI.

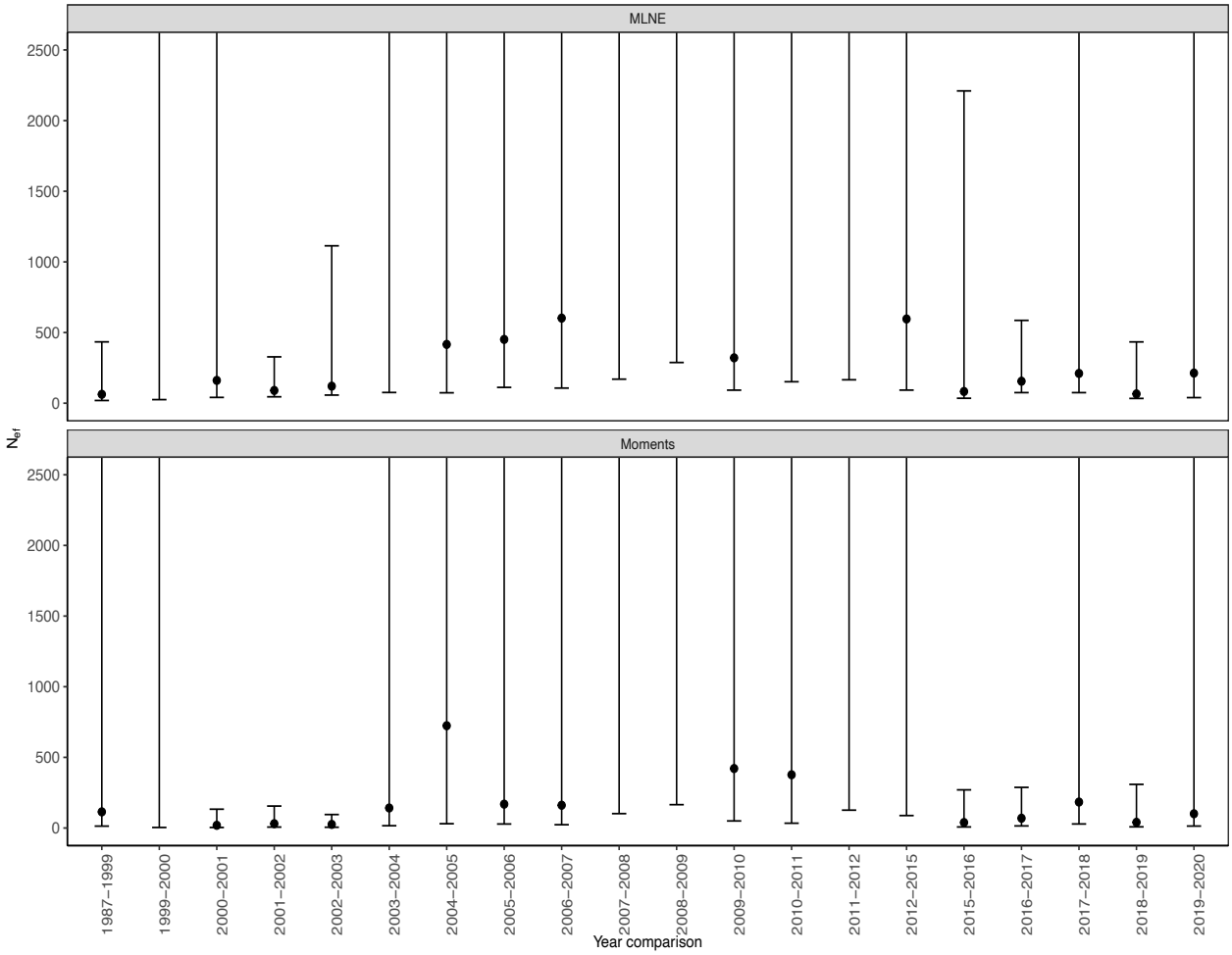

**Figure 6.** Estimates of inbreeding effective size ($N_{eD}$) and their associated 95% confidence intervals. Note the logarithmic scale on y-axis. Infinite mean estimates are indicated by points lying at y-maximum, and upper error bars extending to y-maximum indicate infinite upper bounded 95% CI.