GENETIC MONITORING OF THE RIO GRANDE SILVERY MINNOW: GENETIC STATUS OF WILD AND CAPTIVE STOCKS IN 2015

Annual report FY 2015

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EXECUTIVE SUMMARY

We have conducted genetic monitoring of the Middle Rio Grande population of Rio Grande silvery minnow annually from 1999-2012 and resumed monitoring 2014 and continued in 2015. This work included monitoring stocks that were bred or reared in captivity and were released to the Rio Grande in New Mexico since 2002; when the augmentation program commenced. In 2014, genetic monitoring was not conducted on wild silvery minnow (untagged) because extremely low densities resulted in insufficient samples for genetic analysis; however, monitoring was conducted in 2014 on the hatchery-released members of the Middle Rio Grande population (WCH, tagged), wild caught eggs (WCE), and progeny of captive stocks. Monitoring in 2015 was based on genotyping 143 wild silvery minnow collected in all three occupied reaches of the Middle Rio Grande, as well as wild-caught hatchery released fish (WCH, n=300), and progeny of captive stocks from Southwestern Native Aquatic Resources and Recovery Center (ARRC), the Los Lunas silvery minnow refugium, and the Albuquerque Biological Park.

Major findings for 2015

(1) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies, decreased over values recorded for the previous temporal comparison (2011-2012) regardless of the method used to calculate N_{eV} ranging from 138 to 258. Small estimates of effective size are not surprising given the extremely low densities of Rio Grande silvery minnow in the wild seen between 2010 and 2014; with 2014 densities the lowest since population monitoring began (Dudley et al. 2014). Variance effective size is based on the change in allele frequencies among temporal samples. It is likely that the low N_{eV} estimates seen in 2012-2015 comparison reflect near complete population turnover of Rio Grande silvery minnow. A situation which could have occurred when wild (untagged) silvery minnow reached low densities in 2014, followed by supplementation with fish from captivity, thereby causing substantial shifts in allele frequencies. The rate of loss of genetic diversity is inversely proportional to the genetic effective population size; specifically, where effective population size is very small (i.e. in Rio Grande silvery minnow), the rate of loss of genetic diversity is likely to be high. The theoretical limit for preventing loss of adaptive genetic variation is N_e=500 (Lande 1995).

(2) The linkage disequilibrium estimate of effective size was the smallest (N_{eD}=468) recorded for the wild (untagged) Rio Grande silvery minnow and was similar to that recorded in 2004 (N_{eD}).

Interestingly, both of these temporal samples followed 4-5 years of extremely low abundance and a large input of hatchery reared fish. There are a number of theoretical and practical distinctions between N_{el} (to which N_{eD} estimates are most closely associated) and N_{eV}. These two measures of effective size should be similar in stable populations but show predictable differences in declining (or growing) populations. In declining populations N_{el} 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 and Luikart 2007); therefore, N_{el} is only reduced once mating between close relatives becomes more common (i.e., homozygosity increases in the population). Like N_{ev}, N_{eD} estimates are below the theoretical limit of N_e=500, that the conservation literature suggests is necessary to prevent loss of neutral genetic diversity (Lande 1995).

(3) Mitochondrial DNA (mtDNA) was also used to estimate variance effective population size using a maximum likelihood approach (MLNE). Haplotype frequencies of wild (untagged) fish differed substantially during the period 2012 – 2015, as might occur with a large shift in demographics. Hence, the MLNE estimate of female effective size was considerably smaller (N_{ef} =596) than in the previous temporal comparison (2011 – 2012) in which N_{ef} was indistinguishable from infinity.

(4) All 2015 measures of genetic diversity at microsatellite loci (with all river reaches combined) for wild silvery minnow in the middle Rio Grande were similar to those reported for 2012. This stability is likely the result of the augmentation of the wild population with hatchery produced fish acting to buffer the population against loss of diversity. Average number of alleles (estimated by resampling to account for differences in sample size) has remained stable since 2006. Mitochondrial gene diversity and haplotype richness decreased slightly in 2015 compared the last genetic sample of wild (untagged) individuals (2012) but was within the range seen in previous years. During population bottlenecks, genetic drift causes the loss of rare alleles. Across all samples (including hatchery collections) only the most common eight haplotypes were detected suggesting loss of haplotypes through genetic drift.

(5) Comparisons between WCH fish collected in 2015 and 2014 revealed slightly higher allelic and haplotype diversity in 2015 but lower gene diversity and heterozygosity. The exceptionally low densities of wild fish seen in 2014, suggest that the breeding population in 2014 likely comprised predominantly hatchery bred/reared individuals and as such, the 'wild' fish collected in 2015 reflect genetic diversity and allele frequencies contained in the hatchery stocks (released in 2014). This is precisely what our results indicate, with no significant difference between the WCH 2014 samples and the wild samples taken in 2015. The years 2014 and 2015 were the first years that we included samples of WCH fish for genetic analysis. In previous years

(and in 2014 and 2015) genetic analysis was conducted on the hatchery produced fish before they were released to the river)

(6) In all three river reaches genetic variance effective sizes for wild (untagged) silvery minnow declined from values recorded in 2011-2012. The Isleta reach had the highest effective size (N_{eV}=207, CI 98-1184) compared to both the Angostura (N_{eV}=137, CI 87-238) and San Acacia reaches (N_{eV}=132, CI 73-334). All metrics of genetic diversity were lowest in the San Acacia reach and highest in the Isleta reach. Allelic diversity (N_{ac}=12.32) declined in the San Acacia reach in 2015 compared to values seen in 2012 (N_{ac}=13.30). Low effective size in the San Acacia reach likely reflects the changes in population size associated with the extent of river drying in this reach. Although drying also occurs in the Isleta reach, the population residing here may be buffered somewhat by the reproductive contributions from fish in the Angostura reach that typically remains wet during the summer. Like previous years, there was no genetic structuring by river reach as expected given the drifting nature of Rio Grande silvery minnow eggs.

(7) In fall 2014, <250,000 Rio Grande silvery minnow reared in captivity were released to the Middle Rio Grande and we genotyped representatives from seven lots, from Southwestern Native ARRC, Albuquerque Biopark and Los Lunas Refugium. Five of these lots were the result of captive spawning and two were reared from wild-caught eggs from the Angostura and San Acacia reaches. Stocks originating from the wild had higher average effective population size as estimated using the linkage disequilibrium method than most of the stocks produced by captive spawning. This highlights the importance of collection of eggs from natural spawning events even when the population is extremely low and reiterates findings from previous years.

INTRODUCTION

Genetic monitoring is defined as collection of two or more temporally spaced genetic samples from the same population (Schwartz et al. 2007). Genetic monitoring studies typically employ neutral genetic markers, such as nuclear microsatellites and occasionally maternally inherited mitochondrial DNA (mtDNA), to track changes in diversity metrics over a contemporary time series. Microsatellites are short tandemly repeating DNA sequences that are found throughout the genome of most species (reviewed in Dowling et al. 1996). Microsatellites are biparentally inherited and typically highly variable among individuals (including in highly endangered species and in small populations) and hence are the most widely used genetic markers in molecular ecology and conservation genetics studies. Mitochondrial DNA is a haploid marker (i.e., individuals only have one copy as opposed to two copies for microsatellites), specifically progeny inherit a single mtDNA molecule from their mother. Due to the differences in how nuclear DNA and mtDNA are inherited, they provide complementary approaches to monitoring genetic diversity. The time-scale of genetic monitoring also varies considerably from a sampling over only a few years to the use of archival samples for a monitoring program that may span decades. In these latter studies; that encompass multiple decades, sampling is rarely conducted on an annual basis so linking changes in diversity metrics to specific environmental or management actions may not be plausible. In fish, genetic monitoring to date has been confined largely to marine species and in freshwater systems, such studies primarily involve salmonids. To our knowledge, the data set that we have collected for Rio Grande silvery minnow over the past 17 years represents one of the longer genetic monitoring time series for a non-salmonid freshwater fish. The population is sampled throughout its current range (mean annual sample size = 305), using nine microsatellite loci and a mitochondrial DNA gene to measure changes in various metrics of genetic diversity including allelic richness, heterozygosity, and genetically effective population size (N_e). The temporal component and sampling strategy provides the framework necessary to examine impacts of changes in abundance, management actions and environmental conditions on genetic diversity.

In fishes that are characterized by a short lifespan (the population is dominated by age-1 fish; Horwitz et al. 2011) and in which dramatic changes in abundance occur from year to year (Dudley et al. 2011), negative genetic impacts to the population can occur over relatively short periods. Thus, for species such as Rio Grande silvery minnow, genetic monitoring is a crucial component to management. For example, monitoring data for Rio Grande silvery minnow has i) demonstrated that the genetic effective size is orders of magnitude lower than the census size, ii) shown that the downstream movement of eggs, larvae, and adult fish, and the stocking of the population with captive reared fish, prevented significant divergence of allele frequencies among fishes collected in each of the three river reaches, iii) demonstrated that the diversity of

the wild component of the population was best represented in captive stocks derived from collections of eggs that were produced by natural spawning events in the wild (Osborne et al. 2012); and iv) shown that when artificial breeding is necessary, a group spawning design with equalized (i.e., 1:1) sex ratio produces fish that have levels of diversity that are statistically equivalent to that achieved through a paired mating design (Osborne et al. 2013). These findings have informed the Recovery Plan for the species and have been instrumental in the development of the captive propagation and genetics management plan (USFWS 2009, 2013). Here, we report on the genetic status of the population in 2015 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. 'Wild' fish may have parents that were wild or bred/reared in captivity, but were hatched in the Rio Grande. In 2015, 143 unmarked (i.e. 'wild') silvery minnow were collected. These samples add to the data collected from wild Rio Grande silvery minnow sampled from the Middle Rio Grande annually from 1999 to 2012 (between December 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). We also collected 300 VIE tagged fish from the Middle Rio Grande. These fish represented fish released (from all breeding facilities) in the fall of 2014. The 'wild caught hatchery' (WCH) designation refers to fish hatched and reared in captivity and released into the wild to supplement the Rio Grande silvery minnow population (and its reproduction) in the middle Rio Grande in fall 2014. Sampling for WCH fish commenced in 2014 because of inadequate captures of wild 'untagged' fish. 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). Rio Grande silvery minnow were collected by seining and occasional backpack electrofishing. In 2015, wild fish were collected representing all three river reaches; Angostura (n=75), Isleta (n=33) and San Acacia (n=35). 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. Fish were allowed to recover in untreated river water prior to release.

Sampling- Hatchery Lots

In 2015, fin clips from seven captive lots provided to us by Southwestern Native ARCC (1), the ISC Los Lunas Refugium (1) and the Albuquerque Biological Park (5) totaling 439 individuals were also genotyped. These collections add to the 53 captive stocks sampled previously (2000-2012 and 2014). Mortality from fin-clipping and VIE-tagging is negligible (Southwestern Native ARCC and NMFWCO staff, pers. comm.).

Molecular methods- microsatellites

Total nucleic acids, including genomic DNA and mitochondrial DNA (mtDNA) 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 $MgCl_2$, 125µM dNTPs, 0.40-0.50 µM each primer, 0.375 units *Taq*); and *Ppro 118*/*Ppro126* (1X PCR buffer, 3 mM $MgCl_2$, 125µM dNTPs, 0.40-0.50 µM each primer, 0.375 units *Taq*). *Ca8* was amplified alone (1X PCR buffer, 3 mM $MgCl_2$, 125µM 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 3100 automated capillary sequencer was performed by combining 1 µl of PCR product with 10 µl of formamide + 0.4 µl of HD400 size standard, 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 from each individual was amplified in a 10 µL reaction containing 1 µL template DNA, 1 µL 10× reaction buffer, 2 mM $MgCl_2$, 125 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 U *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

Table 1. Number of wild samples collected from the Rio Grande by year, site and river reach (Angostura, Isleta and San Acacia). Dash mark indicates no samples were collected. Samples collected on 2014 were not analyzed because of the small number 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
Total	**1414**	**1535**	**1610**	**4559**

Table 2. Sample sizes and collection localities of wild Rio Grande silvery minnow by river reach for samples collected during 2015 genetic monitoring.

Sample locations	Angostura	Isleta	San Acacia	Grand Total
Bernalillo	2			2
Rio Rancho	10			10
Alameda	7			7
0.25 mi. upstream Central Ave.	1			1
Central Ave. Bridge	39			39
Avenida Cesar Chavez	15			15
Rio Bravo Blvd. Bridge	1			1
Los Lunas		9		9
Belen		1		1
Jarales		17		17
Bernardo		3		3
La Joya		3		3
SADD			27	27
1.5 mi. downstream of SADD			2	2
Socorro			1	1
3 mi. upstream of US HWY 380 bridge			1	1
8 mi. downstream of San Marcial RR bridge			1	1
10 mi. downstream of San Marcial RR bridge			3	3
Grand Total	**75**	**33**	**35**	**143**

Table 3. Sample sizes and collection localities of wild caught hatchery (WCH) Rio Grande silvery minnow by river reach for samples collected during 2015 genetic monitoring. Also reported are VIE colors (G = green, P = pink, W = white, Y = yellow) and body location (LD = left dorsal, RD = right dorsal) of tagged individuals recovered at each collection locality.

	Angostura				Isleta				San Acacia			
Row Labels	**GLD**	**PRD**	**WLD**	**YLD**	**GLD**	**PRD**	**WLD**	**YLD**	**PRD**	**WLD**	**YLD**	**Grand Total**
Alameda				30								30
0.25 mi. upstream Central Ave.	1	1	1	19								22
Central Ave. Bridge	4	14		8								26
Avenida Cesar Chavez	1	26		12								39
Isleta DD						1						1
Peralta Drain Return					1			14				15
US Hwy 6 bridge crossing						1						1
Alejandro Gate								1				1
NM St HWY 346 Bridge							55	1				56
2 mi. downstream Hwy 346 Bridge					3		27	1				31
Bernardo							4	3				7
Rhodes Property near San Antonio											25	25
SADD									4	2	4	10
1.5 mi. downstream of SADD											1	1
6 mi. upstream of US HWY 380 bridge											24	24
3 mi. upstream of US HWY 380 bridge											10	10
1 mi. upstream of US HWY 380 bridge											1	1
Grand Total	**6**	**41**	**1**	**69**	**4**	**2**	**86**	**20**	**4**	**2**	**65**	**300**

seconds. Nucleotide sequence variation among individual fragments was visualized with single-strand conformational polymorphism (SSCP) analysis (Sunnucks et al. 2000), and representative haplotypes from each gel (~ 20%) were verified by direct sequencing using an ABI 3100 DNA Sequencer.

Statistical analysis

MICROSATELLITE TOOLKIT (add-in for Microsoft Excel, written by S. Park, available at http://animalgenomics.ucd.ie/sdepark/ms-toolkit/) was used to estimate microsatellite diversity estimates of observed heterozygosity (H_o), Nei's unbiased gene diversity (H_e), and mean number of alleles (N_a) as well as to check for microsatellite allele scoring errors . GENEPOP (Raymond and Rousett 1995) 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 For each microsatellite locus and population, inbreeding coefficients (F_{IS}) were obtained using FSTAT vers. 2.9.3.1 (Goudet 1995). Estimates of mtDNA diversity of unbiased haplotype diversity (*h*) were obtained using ARLEQUIN vers. 3.11 (Excoffier et al. 2005). Haplotype richness (H_R) (Petit et al. 1998) was obtained using the program CONTRIB vers.1.02 (available at http://www.pierroton.inra.fr/genetics/labo/Software/Contrib/), which uses a rarefaction approach to correct for unequal sample sizes. The number of haplotypes (N_h) at a locus is also reported for mtDNA (not corrected for difference in sample size). 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.

In some cases, sample sizes differed between collections, particularly between some samples collected early in the study and those collected more recently. Because number of alleles and heterozygosity are dependent on sample size, 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). Diversity estimates (corrected number of alleles [N_{ac}], gene diversity [H_{ec}] and heterozgosity [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 package (www.r-project.org). This resampling techniques was also used for comparisons among collections obtained across years and river reaches, we repeated

the resampling procedure for microsatellite data in *R* where diversity measures were based on n=15 (2004, Isleta) and the smallest sample n=6 (2004, San Acacia) was excluded.

F-statistics

Weir and Cockerham's (1984) *F*-statistics (microsatellites) and Φ-statistics (mtDNA) were calculated in Arlequin ver. 3.11 (Excoffier et al. 2005). 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, WCE, captive-spawned, and captive-reared stocks [i.e. wild-caught eggs [WCE] were the source] (F_{CT}, Φ_{CT}), among samples within these three groups (F_{SC}, Φ_{SC}) and among all samples (F_{ST}, Φ_{ST}). P-values for all statistics were generated using bootstrapping (1000 permutations), as implemented in Arlequin.

Estimation of genetic effective size

Variance genetic 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 (N_{eV} and 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; therefore, calculations of N_{eV} required an estimate of census size (N_c). No reliable, long-term data (i.e., spanning the entire sampling period) were available for N_c, so each pairwise comparison was run under the following two N_c scenarios: a "crashed" (N_c = 10,000) and a "large" (1,000,000 individuals) population. The former value is lower than any census size estimate to date and the latter is within the order of magnitude for which larger N_c have been recorded (Dudley et al. 2011). In all comparisons, differences in mean N_{eV} were negligible between the N_c = 10,000 and N_c = 1,000,000 scenarios, but lower and upper confidence intervals were slightly larger for the latter. Only the most conservative N_{eV} estimates (i.e., based on N_c = 1,000,000) are reported herein. Jackknife estimation over all loci was used to calculate N_{eV} and associated 95% confidence intervals.

For all methods we assumed that migration (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_e 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 the estimates of N_{eV}, which requires samples from different time periods, we used the linkage disequilibrium method (N_{eD}, Hill 1981), that only requires a single sample. N_{eD} was estimated from microsatellite DNA data for each annual sample (including wild, wild caught hatchery [2014 only], captive-spawned and wild-caught eggs), using the program NEESTIMATOR (Do et al. 2014) and methods described in Osborne et al. (2010). 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).

For mtDNA data, variance effective size for the female portion of the population (N_{ef}) was estimated with temporal (Turner et al. 2001) and pseudo-maximum-likelihood (MLNE) methods. TEMPOFS was not used for mtDNA data as this method assumes diploidy (Jorde and Ryman 2007). It is useful to estimate genetic effective size from mitochondrial DNA data because it provides information about what the female portion of the population is doing. For example, if very low estimates of N_{ef} were obtained it would suggest that very few females are making a genetic contribution to the population.

RESULTS

Microsatellites- genetic diversity

In 2015, we sampled 443 Rio Grande silvery minnow, of which 143 individuals were wild and 300 were WCH fish (Tables 1 to 3). An additional 439 individuals produced in captivity were provided to us for analysis. Within the three reaches of the middle Rio Grande, 75 wild and 117 WCH were collected from the Angostura reach, 33 wild and 112 WCH from the Isleta reach, and 35 wild and 71 WCH from the San Acacia reach (Tables 2 and 3). To date, we have now genotyped a total 7,149 individuals at nine microsatellite loci representing wild fish across 17 years and hatchery stocks.

Characterization of microsatellite genotypes from the 2015 samples revealed two loci (*Ca6* and *Ppro126*) as the least variable, each with eight alleles detected across all samples. Locus *Ppro118* was the most variable with 62 alleles followed by *M1* with 41 alleles. Tests for deviations from Hardy-Weinberg Proportions were significant for 49% of locus-by-site combinations (40/81) at the nominal alpha level of 0.05. However, only 33% of comparisons were significant following sequential Bonferroni correction for multiple comparisons. Significant departures were primarily concentrated to four loci (*M7*, *M8*, *Ca8*, *Ppro118*), for which analysis by MICRO-CHECKER (Van Oosterhout et al. 2004) found an excess of homozygous individuals which could be due to the presence of null alleles. Significant tests for genotypic disequilibrium occurred in 22 of 324 comparisons following sequential Bonferroni correction and all but two of these occurred in captive stock samples

Microsatellite diversity, characterized by corrected values of mean number of alleles (N_{ac}) Nei's unbiased gene diversity (H_e), and observed heteroygosity (H_o), for wild fish sampled from the Middle Rio Grande in 2015 was within the minimum and maximum values reported for all wild fish collected since genetic monitoring began. Both wild and WCH fish had similar levels of genetic diversity at microsatellite loci after correcting for differences in sample size (Table 4). Genetic diversity estimates of wild and WCH fish were also most similar to captive lot from Albuquerque BioPark released in 2014. This lot also contained the greatest diversity compared to all captive stock samples. Across all stocks, diversity estimates were lowest in samples taken from the Los Lunas Refugium.

mtDNA- genetic diversity

Mitochondrial diversity was characterized by number of haplotypes (N_h), haplotype diversity (h), and haplotype richness (H_R). These metrics are equivalent to the number of alleles, gene diversity (H_{ec}), allelic diversity (N_{ac}) averaged across microsatellite loci. A total of 17 mtDNA haplotypes have been identified from assaying 7340 wild (untagged) individuals from the Middle Rio Grande from 1987 to 2015 (Table 4; Osborne and Turner 2012). Haplotype A was the most common in all samples except Cs-An-02 (captive spawned in 2002), which was monomorphic for Haplotype D. Three haplotypes (C, D, F) were present at moderate frequencies (>5%) and 11 haplotypes were uncommon (<5%) or rare <1%). Across the time series, haplotype diversity was highest in the 1987 sample (h = 0.743) and lowest in 2000 (h = 0.364). In 2015, eight haplotypes (A, C, D, E, F, K, M and O Table 5) were observed among all collections with haplotype A the most common, followed by haplotypes C and D. Haplotype diversity ranged from 0.41 (ABP14-001) to 0.67 (ABP13-006 and Los Lunas), with a value of 0.63 for both wild and WCH; numbers of haplotypes and haplotype diversities observed in 2015 samples were typical of those from previous years (Figure 1; Table 4).

Microsatellites- population structure

Total population structure was evaluated using global F_{ST} estimates across all 2015 samples, including hatchery stocks, and although F_{ST} was significantly different from zero it was very small (F_{ST} = 0.007, 95% CI = 0.006 to 0.009). Pairwise F_{ST} was estimated between samples collected in each river reach to assess genetic structure among wild fish. Consistent with analyses in previous year, no significant values were observed indicating no genetic structuring by reach. Pairwise F_{ST} was also estimated between all 2015 wild fish (reaches combined) and captive stocks as well as WCH fish collected in 2014 to identify the possible source of the wild fish. Comparisons between wild fish were non-significant with all three Albuquerque BioPark 2014 lots as well as two samples of WCH collected in 2014. F_{ST} values between wild fish and Southwestern Native ARRC (0.004) and Los Lunas (0.011) were significant but small.

mtDNA- population structure

Φ-statistics, among river reaches, were calculated for 2015 wild Rio Grande silvery minnow. Genetic differences among the Angostura, Isleta, and San Acacia reaches were not significant (Φ_{CT} = -0.001, P = 0.812).

Genetic effective size- microsatellites

Moments, TEMPOFS , and MLNE estimates of variance effective size, N_{eV}, from microsatellites, are shown in Figure 2. For 2012-2015, estimates were N_{eV} = 229 (moments), N_{eV} = 138 (TEMPOFS, 95% CI 93-203), and N_{eV} = 258 (MLNE, 95% CI 205-336). All estimates were significantly lower than those reported by Osborne and Turner (2012) for the 2011-2012 period (moments, N_{eV} = 462 (95% CI, 286-855); TempoFS, ∞ (95% CI, 9829-∞); and MLNE, N_{eV} = 803 (95% CI, 527-1523). MLNE and moments estimates of female variance effective size, N_{ef}, based on mtDNA are shown in Figure 3. From 2012-2015, estimates of N_{ef} were infinite (moments) and N_{ef} = 596 (MLNE, 95% CI 93-infinity).

Estimates of inbreeding effective size (Figure 4; Table 4) were N_{eD} = 468 (95% CI 281-1189) for wild and N_{eD} = 289 (95% CI 206-443) for WCH fish collected in 2015. For captive stocks released in the middle Rio Grande in fall 2013 from Albuquerque BioPark, estimates were N_{eD} = 36 (95% CI 28-49) and N_{eD} = 27 (95% CI 22-33). Estimates from the three Albuquerque BioPark stocks released in 2014 were larger and ranged from N_{eD} = 133 (95% CI 86-262) to N_{eD} = 194 (95% CI 115-535). Effective size of captive stocks from Southwest Native ARRC, N_{eD} = 179 (95% CI 127-279), were similar to the BioPark, whereas Los Lunas Refugium had the smallest effective size N_{eD} = 21 (95% CI 18-25). Estimates of N_{eD} for wild, WCH, and captive stocks were significantly lower than that observed in wild fish from 2012 (N_{eD} = 10,064; Figure 4 and Table 4) and were the lowest estimates observed since genetic monitoring began.

Table 4. 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. No genetic monitoring was conducted in 2013. Values from 2015 monitoring year are bolded 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	14.00	0.797	0.710	0.111	∞	139	∞	37	7	0.74	6.00
1999	46	12.23	0.814	0.647	0.210	∞	∞	∞	44	5	0.43	3.82
2000	194	14.33	0.814	0.697	0.145	∞	∞	∞	124	6	0.36	3.36
2001	128	15.01	0.807	0.721	0.107	2008	495	∞	122	10	0.61	6.06
2002	389	14.75	0.793	0.681	0.143	1951	702	∞	387	8	0.63	4.16
2003	169	14.95	0.817	0.709	0.134	2998	564	∞	167	9	0.52	4.89
2004	162	14.85	0.819	0.737	0.100	596	357	1559	161	10	0.62	6.28
2005	394	14.90	0.816	0.724	0.113	2724	1014	∞	396	10	0.61	5.63
2006	383	15.26	0.826	0.727	0.122	2562	1291	34064	378	10	0.62	5.67
2007	218	15.08	0.828	0.726	0.123	∞	1211	∞	218	10	0.58	5.36
2008	474	15.16	0.823	0.713	0.135	4459	1479	∞	466	11	0.57	5.30
2009	476	15.11	0.830	0.689	0.172	3608	1677	∞	472	12	0.59	5.65
2010	440	15.19	0.834	0.692	0.172	∞	2023	∞	433	9	0.65	6.09
2011	362	15.32	0.830	0.724	0.130	∞	3117	∞	359	11	0.63	5.74
2012	517	15.42	0.827	0.728	0.123	10064	1782	∞	522	11	0.66	5.68
2013	-	-	-	-	-	-	-	-	-	-	-	-
2014*	12	-	-	-	-	-	-	-	-	-	-	-
2015	**144**	**15.33**	**0.815**	**0.731**	**0. 103**	**468**	**281**	**1189**	**143**	**8**	**0.63**	**5.42**

WCH	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	h	H_R
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**

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
2013	-	-	-	-	-	-	-	-	-	-	-	-
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**

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	-	1.00

Captive spawned (cont.)	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	h	H_R
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	0.70	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	14.70	0.825	0.727	0.121	323	134	∞	45	5	0.66	4.79
TFT_04_31	50	12.80	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	5.80
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	12.90	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
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	0.7	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	4.60
LL_12	49	12.48	0.794	0.684	0.141	41	33	52	48	6	0.63	4.49

Captive spawned (cont.)	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	h	H_R
MJO_12_09	50	14.03	0.829	0.721	0.133	62	46	88	49	4	0.60	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	14.70	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**	**12.90**	**0.799**	**0.703**	**0.122**	**27**	**22**	**33**	**50**	**3**	**0.50**	**2.00**
ABP14_004	**49**	**13.90**	**0.807**	**0.683**	**0.155**	**133**	**86**	**262**	**49**	**8**	**0.56**	**6.01**
CSDX14_SNARRC	**150**	**14.65**	**0.827**	**0.728**	**0.120**	**179**	**127**	**279**	**147**	**7**	**0.6**	**4.49**
CSDX14_LL	**55**	**11.66**	**0.789**	**0.744**	**0.058**	**21**	**18**	**25**	**52**	**3**	**0.67**	**2**

Table 5. 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 2015 monitoring year are bolded for emphasis.

	mtDNA haplotypes																
Wild-MRG	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	W	V
1987	45.9	16.2	16.2	5.4	8.1	2.7	-	-	5.4	-	-	-	-	-	-	-	-
1999	75.0	-	11.4	6.8	4.5	2.3	-	-	-	-	-	-	-	-	-	-	-
2000	79.0	0.8	4.8	4.8	9.7	0.8	-	-	-	-	-	-	-	-	-	-	-
2001	60.7	9.0	5.7	3.3	9.8	7.4	0.8	1.6	0.8	-	0.8	-	-	-	-	-	-
2002	55.6	19.9	13.7	1.0	5.9	3.4	-	0.3	-	-	0.3	-	-	-	-	-	-
2003	67.1	5.4	15.0	3.0	5.4	1.2	-	0.6	0.6	-	1.8	-	-	-	-	-	-
2004	59.6	8.7	10.6	1.9	7.5	5.0	1.2	-	1.9	-	3.1	0.6	-	-	-	-	-
2005	59.8	12.6	8.8	2.8	8.6	1.8	1.5	0.3	2.8	-	1.0	-	-	-	-	-	-
2006	58.7	13.5	9.3	4.8	4.8	4.8	0.3	-	2.9	-	0.8	-	-	-	0.3	-	-
2007	62.8	11.0	8.3	2.3	8.7	3.7	0.5	-	0.5	-	1.8	-	0.5	-	-	-	-
2008	63.5	12.0	7.9	2.6	6.7	4.5	0.4	-	0.9	-	0.6	0.2	-	0.6	-	-	-
2009	61.4	14.0	7.6	2.8	6.4	3.4	0.6	0.4	1.9	-	1.1	0.2	-	0.2	-	-	-
2010	56.2	12.4	9.7	3.2	6.9	5.3	1.4	-	1.6	-	3.2	-	-	-	-	-	-
2011	57.4	14.2	10.9	2.8	6.4	3.1	0.6	-	3.1	-	1.1	-	0.3	0.3	-	-	-
2012	53.8	16.5	11.6	3.4	7.2	3.0	0.4	0.4	1.7	-	1.7	-	0.2	-	-	-	-
2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2015	**57.0**	**13.0**	**13.0**	**1.0**	**6.0**	**3.0**	**-**	**-**	**1.0**	**-**	**5.0**	**-**	**-**	**-**	**-**	**-**	**-**

	mtDNA haplotypes																
WCH	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	V
2014	57.7	20.9	6.0	-	11.5	0.1	-	-	-	-	3.3	-	-	-	-	-	-
2015	**57.0**	**13.0**	**15.0**	**2.0**	**3.0**	**2.0**	**-**	**-**	**2.0**	**-**	**6.0**	**-**	**-**	**-**	**-**	**-**	**-**

	mtDNA haplotypes																
Wild caught eggs	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	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	7.5	-	1.9	-	-	-	-	-	-	-	-	-
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	-	-	-	-
2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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**	**-**	**-**	**-**	**-**	**-**	**-**

	mtDNA haplotypes																
Captive spawned	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	V
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	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	-	-	-	-	-	-	-	-

	mtDNA haplotypes																
Captive spawned	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	V
TFT_04_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	-	0.0	-	-	-	-	-	-	-	-	-	-	-
TFT_05_08	59.2	8.2	-	10.2	-	22.4	-	-	-	-	-	-	-	-	-	-	-
TFT_05_09	68.0	16.0	-	-	-	12.0	-	-	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	-	-	-	-	-	-	-	5.9
MJO_11_12	64.0	12.0	4.0	-	-	-	-	-	14.0	-	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	-	12.0	-	-	-	-	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**	**-**	**-**	**-**	**-**	**-**	**-**
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**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

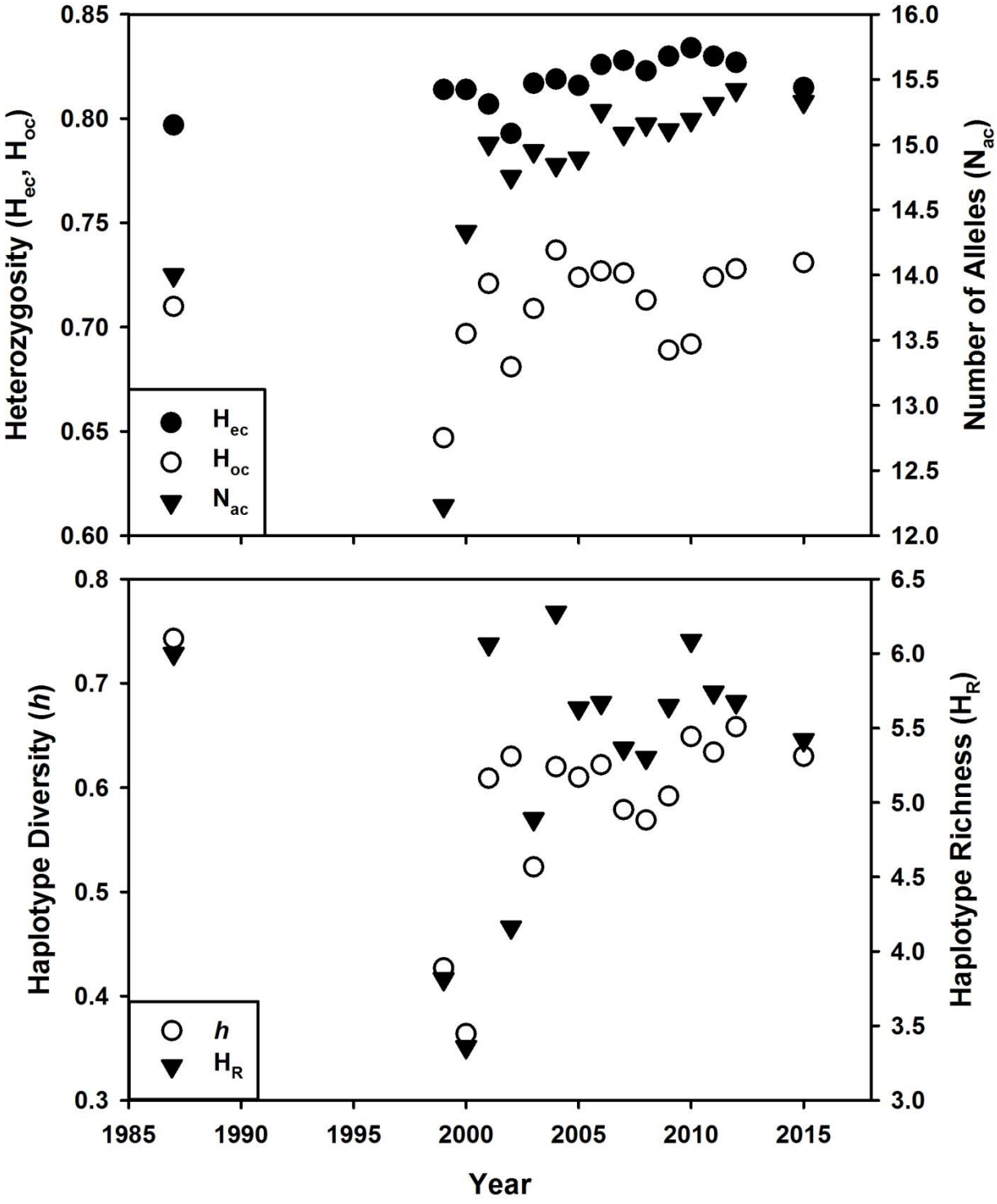

Figure 1. Wild (no VIE tag) Rio Grande silvery minnow diversity metrics based on microsatellite loci (top panel) and mtDNA (bottom) from 1987 to 2015. Diversity estimates were corrected for differences in samples sizes across years using a resampling technique (see Methods

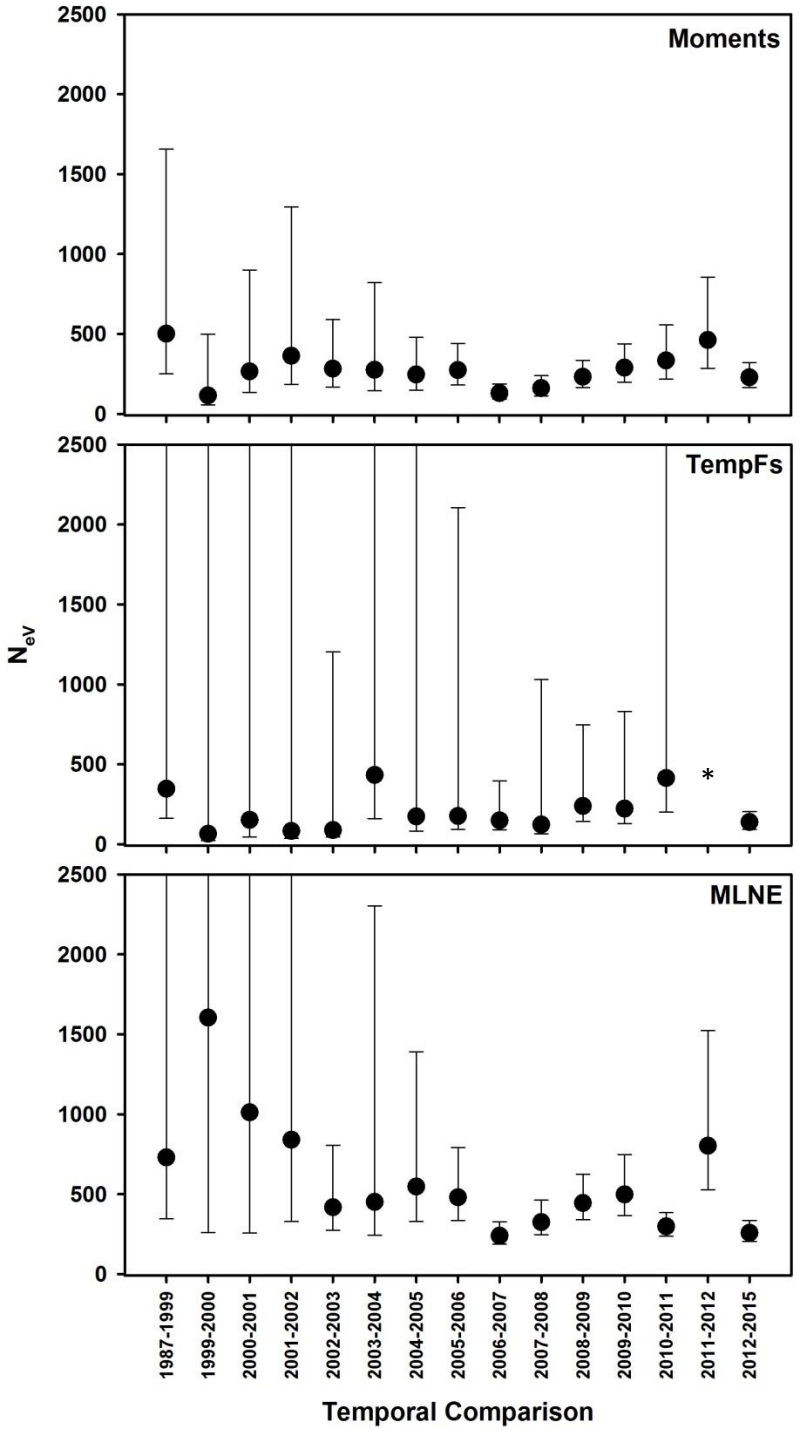

Figure 2. Microsatellite variance effective size estimates (N_{eV}, requiring two temporal samples) for wild (no VIE tag) Rio Grande silvery minnow from 1987 to 2015. N_{eV} was estimated using three methods, moments (upper), TEMPOFS (middle), and MLNE (lower). Error bars represent estimate 95% CIs. Mean TEMPOFS estimate from 2011-2012 (*) was infinite, and upper error bars extending to y-maxima indicate infinite upper bounded 95% CI.

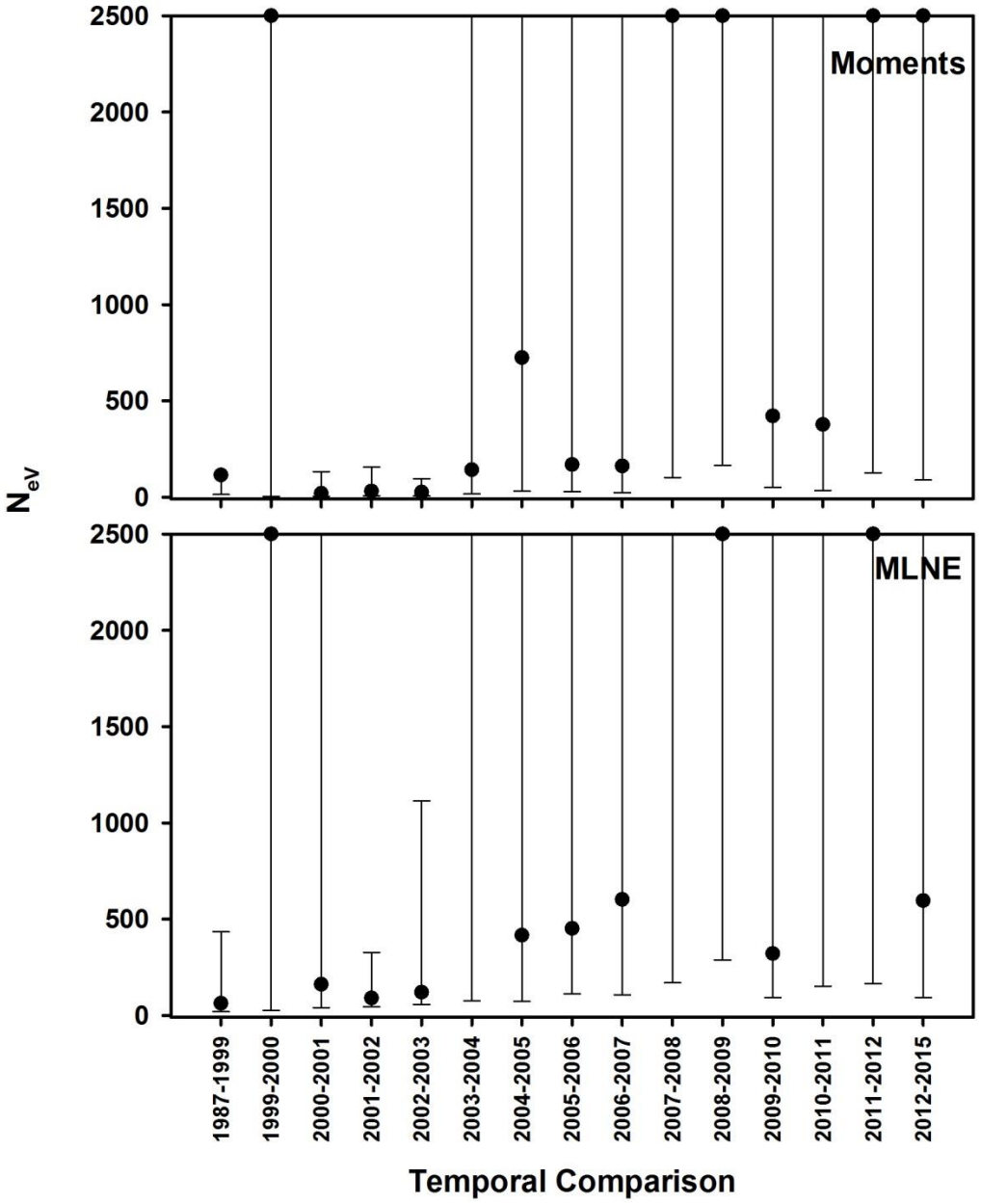

Figure 3. Mitochondrial variance effective size estimates (N_{eV}) for wild (no VIE tag) Rio Grande silvery minnow from 1987 to 2015. N_{eV} was estimated using two methods, moments (upper) and MLNE (lower). Error bars represent estimate 95% Cis. Infinite mean estimates are indicated by points lying at y-maxima and upper error bars extending to y-maxima indicate infinite upper bounded 95% CI.

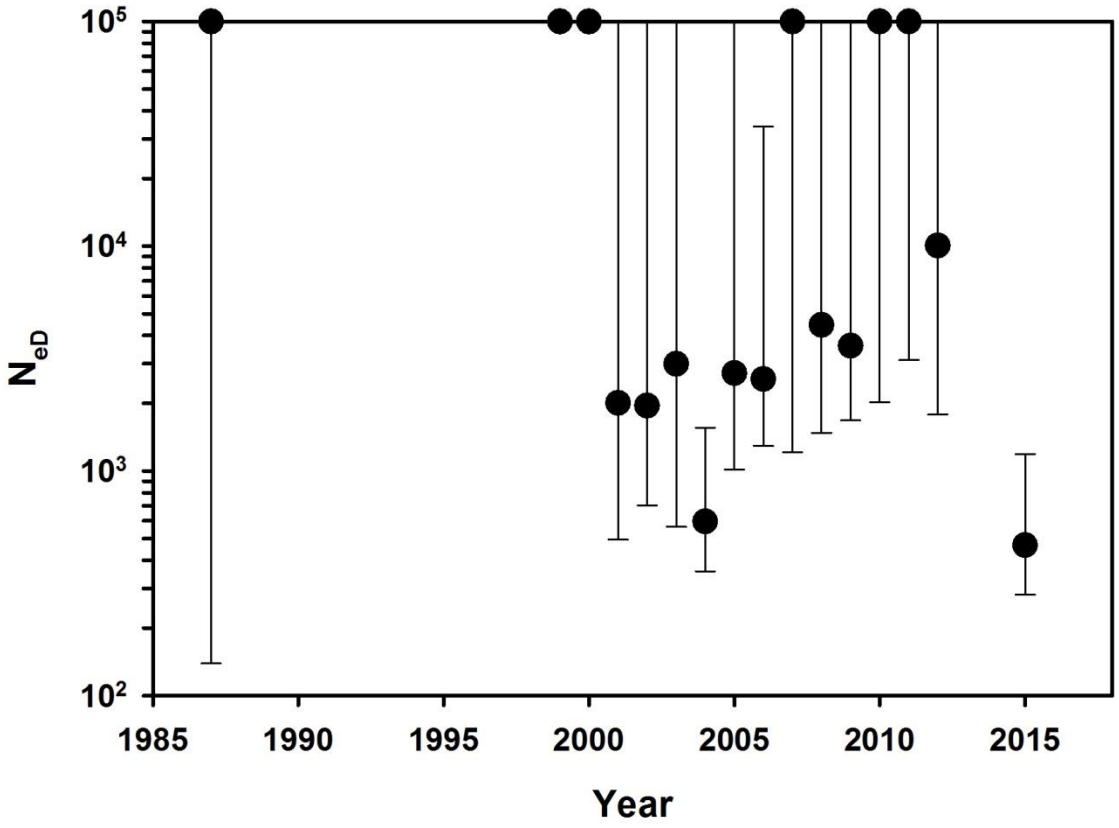

Figure 4. Microsatellite inbreeding effective size based on the linkage disequilibrium method (N_{eD}, single sample) for wild (no VIE tag) Rio Grande silvery minnow from 1987 to 2015. Error bars represent 95% CIs. Note the logarithmic scale on y-axis. Infinite mean estimates are indicated by points lying at y-maximum, and upper error bars extending to y-maximum indicate infinite upper bounded 95% CI.

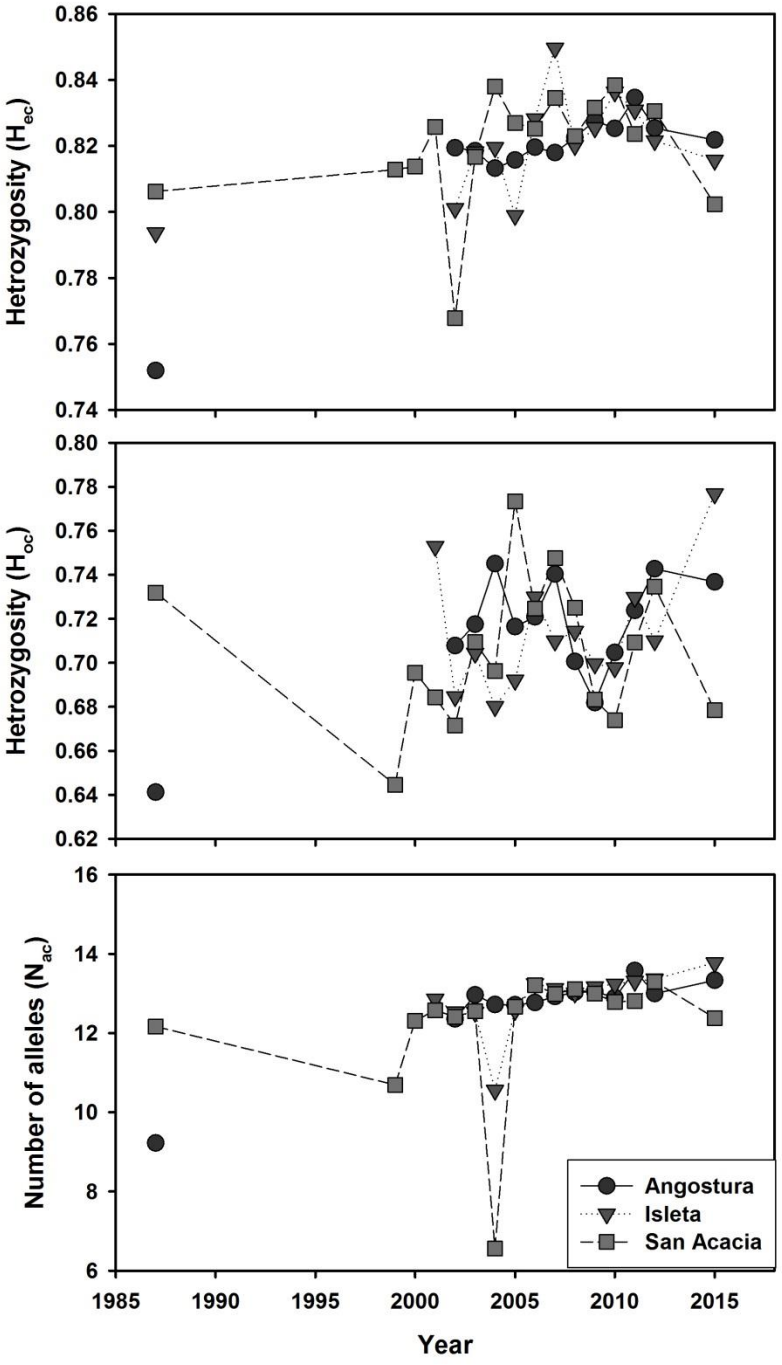

Figure 5. Wild (no VIE tag) Rio Grande silvery minnow diversity metrics separated by river from 1987 to 2015. Microsatellite diversity estimates, H_{ec}, (top) H_{oc}, (middle) N_{ac} (bottom), were corrected for differences in sample sizes across years and river reach by resampling (see Methods).

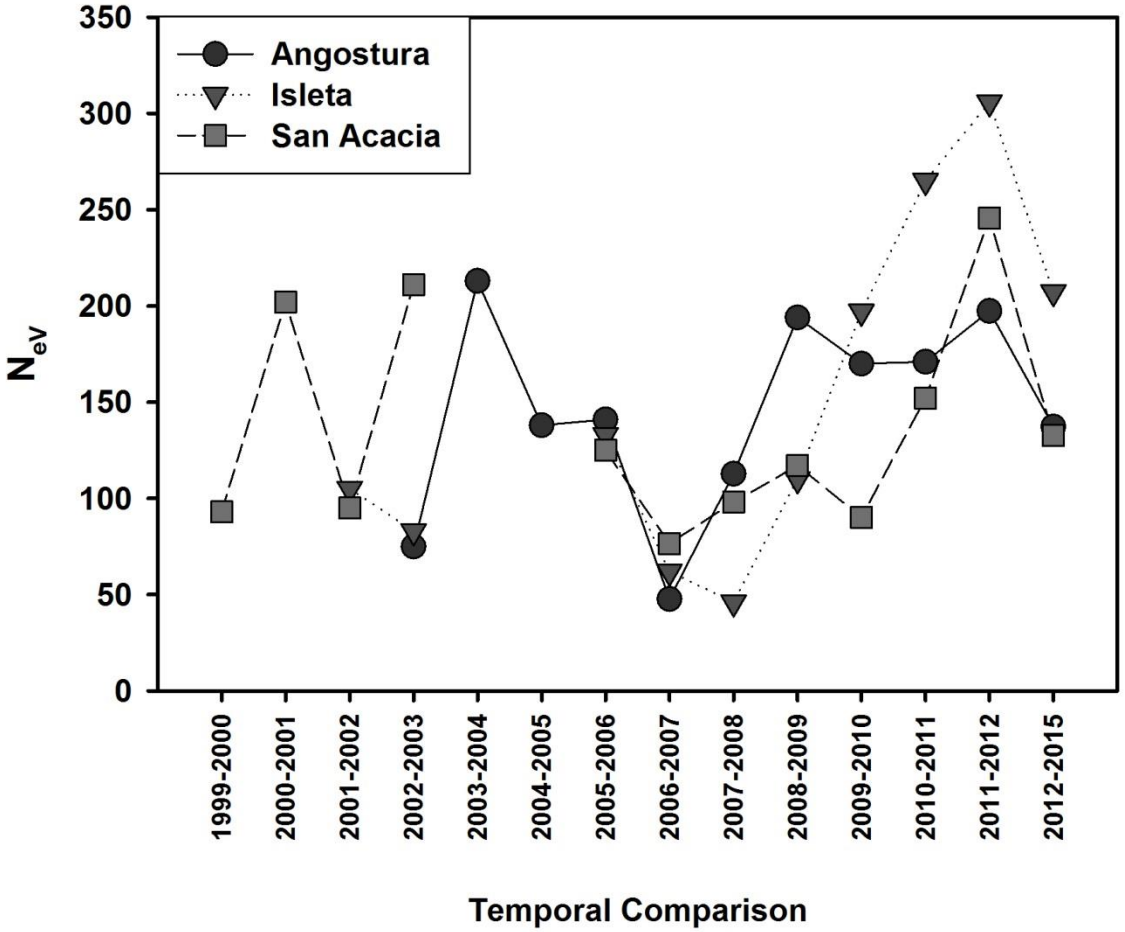

Figure 6. Microsatellite variance effective size estimates (N_{eV}) of wild (no VIE tag) Rio Grande silvery minnow from 1999 to 2015 separated by river reach. N_{eV} estimation methods (moments, TEMPOFS, MLNE) were congruent, thus only results from TEMPOFS method are presented.

DISCUSSION

Status of wild Rio Grande Silvery Minnow in 2015

The population monitoring program for Rio Grande silvery minnow (1993-2015) show that the wild population has experienced multiple, order of magnitude changes in density over the past two decades (Dudley et al. 2014). In particular, the lowest densities of Rio Grande silvery minnow have been recorded during 2012 – 2014 whilst densities from 2010-2014 were substantially lower than the period 2007-2009 (Dudley et al. 2014). Although population monitoring data shows that Rio Grande silvery minnow has the capacity to rebound rapidly following periods of very low density, declines are expected to gradually erode genetic diversity particularly in the absence of actions to buffer the population (i.e. supportive breeding and augmentation). Genetic monitoring data (1999-2012 and 2014) for Rio Grande silvery minnow has shown that despite declines in the population, augmentation with fishes reared in captivity has this far prevented catastrophic loss of diversity. The exceptionally low densities of wild fish seen in 2014, suggest that the breeding population in 2014 likely comprised predominantly hatchery bred/reared individuals and as such the 'wild' fish collected in 2015 will reflect genetic diversity and allele frequencies contained in the hatchery stocks (released in 2014). This is precisely what our results indicate, with no significant difference in allele frequencies (i.e., F_{ST}) between the WCH 2014 samples and the wild samples taken in 2015.

From 1987 and 1999-2004, for both microsatellites and mtDNA there was 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 there were marginal increases in mtDNA and microsatellite diversity. Microsatellite gene diversity declined slightly in 2015 and was most similar to values recorded in 1999-2000 and 2003-2005. However, gene diversity (heterozygosity) is insensitive to large decline in population size because even when a population is reduced to very small size heterozygosity is maintained, unless the bottleneck persists for a number of generations. Allelic richness has remained stable since 2006 and continued to do so in 2015. Mitochondrial haplotype diversity and richness both declined from values seen in 2012. During population bottlenecks, genetic drift causes the loss of rare alleles. Across all samples (including hatchery collections) only the most common eight haplotypes were detected suggesting loss of haplotypes through genetic drift.

Estimates (from microsatellite data) of N_{eV} for the 2012-2015 time period calculated using the temporal method decreased from values calculated for the previous time period (2011-2012). Decreasing N_{eV} suggests changes in allele frequencies from year to year and for 2015 this result

is not surprising, given the likely increased contribution of hatchery fish to reproduction in 2014 (and sampled as adults in 2015) compared to 2012. Similarly, genetic effective size estimated using the linkage disequilibrium method (N_{eD}=468) showed more than an order of magnitude decline when compared to the 2012 estimate (N_{eD}=10,064) and was most similar to the 2004 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_{el} (to which N_{eD} estimates are most closely associated) and N_{eV}. 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_{el} 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 and Luikart 2007); therefore, N_{el} is only reduced once mating between close relatives becomes more common (i.e., homozygosity increases in the population). Values of N_{eD} were uniformly higher than estimates of N_{eV} in previous years and this trend continued in 2015. Estimates of effective size (MLNE) made from mitochondrial DNA haplotype frequency data also decreased for the 2012-2015 time period. Statistical power depends on sample size (which was roughly equal for the two datasets) and the number of independent haplotypes (Waples 1989; Ruzzante et al. 1996). MtDNA represents a single locus (compared to nine microsatellite loci) and has relatively few haplotypes with one predominant haplotype (A) whereas the number of microsatellite alleles is an order of magnitude higher. Therefore, microsatellites have more power to detect changes in allele frequencies than mtDNA.

Reach Specific Findings

All metrics of genetic diversity were lowest in the San Acacia reach and highest in the Isleta reach and genetic effective size estimates (N_{eV}) followed a similar pattern (Figures 5 and 6). Genetic effective size estimates are based on changes in allele frequencies between temporal samples (e.g. fish collected from the San Acacia in 2012 compared to 2015). In population segments where there is high population turnover, allele frequencies may change between temporal samples resulting in low N_{eV} estimates. Low effective size in the San Acacia reach likely reflects the changes in population size associated with the extent of river drying in this reach. Although drying also occurs in the Isleta reach, the population residing here may be buffered somewhat by the reproductive contributions from fish in the Angostura that typically remains wet during the summer.

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

In fall 2014, 268,000 fish were released in the middle Rio Grande New Mexico and five of the lots released were derived primarily from captive spawning. Two lots were reared from eggs

collected from natural spawning in the middle Rio Grande in 2014. These lots had the highest genetic effective sizes and microsatellite diversity was also comparable to the wild population in previous years. These results are consistent with findings in previous years, and highlight the importance of using wild-caught eggs for stocking and refreshment of the captive broodstock. Collection of wild produced eggs helps to preserve rare alleles that may otherwise be lost when captive stocks are derived from relatively few breeders. Eggs collected from natural spawning events should encompass the genetic variation of more breeders than can be accomplished by captive spawning. Mitochondrial haplotype richness (corrected for differences in sample size) was variable across lots and facilities ranging from 2-7. Low haplotype representation in several hatchery lots highlights the importance of spawning large numbers of adults so that rare haplotypes are maintained.

CONCLUSIONS

Seventeen 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 of this study indicate that the trajectory of genetic change in the wild Rio Grande silvery minnow population is determined largely by supplementation with captive reared stocks and not by 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. However in 2015, we saw all measures of genetic effective size fall below the theoretical minimum of N_e=500 necessary to maintain neutral variation and to avoid negative genetic impact associated with inbreeding (Lande 1995). In 2014, like in 2003-2004, the wild population was likely replaced largely by hatchery-bred/reared fish. This highlights the importance of continued monitoring the captive stocks and of the wild population as any detrimental effects (such as losses of diversity) in the captive stocks will ultimately by transferred to the 'wild' population.

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