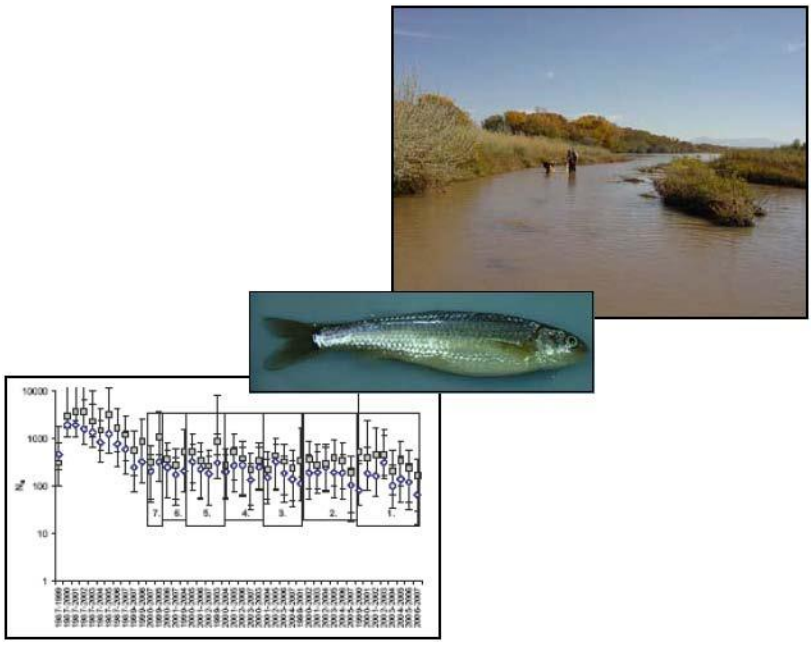
Genetic monitoring of the Rio Grande silvery minnow: Genetic status of wild and captive stocks in 2012.



Annual report FY 2012

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Executive Summary

We have been conducting genetic monitoring of the wild middle Rio Grande population of Rio Grande silvery minnow on an annual basis since 1999. We has also been monitoring stocks of fish bred and/or reared in captivity and then released to the Rio Grande in New Mexico since 2002 and of fish released to Big Bend (TX) since 2008. In 2012, we continued these efforts by genotyping 517 wild fish from the middle Rio Grande, NM, and 700 (representing 14 lots) progeny of captive spawning and/or rearing conducted at Dexter National Fish Hatchery and Technology Center, the Albuquerque Biological Park and the Los Lunas silvery minnow refugium.

Major Findings for 2012:

(1) All measures of genetic diversity at microsatellite loci and mtDNA-ND4 in the middle Rio Grande population were similar to last year. Average number of alleles (estimated by resampling) has remained stable since 2006 and continued to do so in 2012. Mitochondrial gene diversity was similar to values seen in 2010 and 2011 whilst haplotype richness was marginally less than in previous years.

(2) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies, increased over values recorded for the previous temporal comparison regardless of the method used to calculate N_{eV}.

(3) Mitochondrial DNA haplotype frequencies were very similar to those in 2011, hence estimates of female effective size were large and indistinguishable from infinity.

(4) Captive spawned Rio Grande silvery minnow released to Big Bend National Park had comparable levels of microsatellite gene diversity and heterozygosity to the wild population. Mitochondrial gene diversity was also comparable to the wild population with ten haplotype represented in fish released in fall 2011 and 11 haplotypes represented since this population was established in 2008.

(5) In fall 2011, three lots of fish reared from wild-caught eggs were released in the middle Rio Grande. These had higher average number of microsatellite alleles and haplotypes and also had considerably higher average effective population size (>1000 compared to 65) as estimated using the linkage disequilibrium method than fish produced by captive spawning. This highlights the importance of collection of eggs from natural spawning events.

(6) Release of fish in the Angostura reach ceased in 2008 for a trail period of 5 years. Stocking continued in the Isleta and San Acacia reaches. In the Isleta and San Acacia reaches

there has been an upward trajectory of some diversity metrics including haplotype richness and haplotype diversity since 2008. This trend was not seen in the Angostura reach. For microsatellites the average number of alleles increased in the Isleta reach, declined from 2008-2011 and then increased in 2012. In the Angostura reach, the average number of alleles remained essentially the same from 2007-2012 whilst heterozygosity has increased.

(7) There has been a trend of increasing variance effective size in both the Isleta and San Acacia reaches since lows recorded in 2006-2007. In the Angostura reach N_{eV} has remained stable at values of around 200 for all comparisons beginning with 2008-2009.

Introduction

Genetic monitoring is defined as collection of two or more temporally spaced genetic samples from the same population (Schwartz et al. 2007). In fish, genetic monitoring to date has been confined largely to marine species and in freshwater systems, such studies primarily involve salmonids. Genetic monitoring studies typically employ neutral genetic markers, such as microsatellites and occasionally mitochondrial DNA, to track changes in diversity metrics across multiple contemporary time-points. The number of loci employed varies among species with between five and 14 microsatellites used in recently published studies. 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. To our knowledge, the data set that we have collected for Rio Grande silvery minnow over the past 15 years represents the longest genetic monitoring time series for a non-salmonid freshwater fish. The population is sampled throughout its current range (mean annual sample size =278), 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 population diversity.

In species such as Rio Grande silvery minnow that are characterized by their short lifespan (the population is dominated by age-1 fish) and dramatic changes in abundance from year to year, negative genetic impacts to the population can occur over relatively short time scales. For this reason, genetic monitoring is a crucial component to the management of the species. In fact, the data collected as part of the genetic monitoring program for Rio Grande silvery minnow has informed management in the following ways i) demonstrated that the genetic effective size is orders of magnitude lower that the census size, ii) there is not significant divergence of allele frequencies among fishes collected in each of the three river reaches [due to downstream movement of eggs, larvae and adult fish and stocking of the population with captive reared fish], iii) diversity of the wild population is best reflected (in captive stocks) by collection of eggs produced by natural spawning events in the wild, iv) when artificial breeding is necessary, a group spawning design (with equalized sex ratio) produces fish that have levels of diversity that are comparable to that achieved with a paired mating design. These finding have helped to inform the Recovery Plan for the species and were instrumental in the development of the captive propagation and genetics management plan. Here we report on the genetic status of the population in 2012.

Methods

Sampling-Rio Grande Population

Rio Grande silvery minnows were sampled in the Rio Grande annually from 1999 to 2012

Table 1. Sample sizes, collection localities on the Rio Grande, river reaches for wild Rio Grande silvery minnow samples collected for 2012 genetic monitoring.

River	Locality	Sample Size
Angostura	Calabacillas Arroyo	35
Angostura	Sth of Paseo del Norte	50
Angostura	Sandia Line 14	2
Angostura	AMAFCA Channel	9
Angostura	Dixon Rd	3
Angostura	Central Ave Bridge	51
Isleta	Below Isleta DD	16
Isleta	Alejandro Drain	25
Isleta	Upstream Hwy 6	50
Isleta	Isleta Diversion Dam	17
Isleta	Peralta	50
Isleta	Bernardo	52
Isleta	3.5 m downstream Hwy 60	5
San Acacia	San Acacia Diversion Dam	2
San Acacia	San Antonio	2
San Acacia	San Marcial	49
San Acacia	Station 500	52
San Acacia	Egg monitoring site	50

Table 2. Number of wild samples collected from the Rio Grande by year, site and river reach (Angostura, Isleta and San Acacia).

	Angostura	Isleta	San Acacia
1987	15	-	28
1999	-	-	46
2000	-	-	194
2001	-	65	63
2002	67	121	201
2003	71	65	33
2004	141	15	6
2005	190	109	95
2006	95	143	145
2007	48	128	42
2008	165	191	123
2009	175	153	150
2010	149	146	151
2011	71	148	140
2012	147	215	154

(between December and April – just prior to reproduction) (Table 1 and Table 2). In addition, 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) were genotyped. 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. Collections were made throughout the current distribution of Rio Grande silvery minnow that extends from Cochiti reservoir to Elephant Butte reservoir in New Mexico. Sampling was not conducted in the Cochiti reach where the Rio Grande silvery minnow is considered rare (Bestgen & Platania 1991). Rio Grande silvery minnow were collected by seining and occasional backpack electrofishing. Fish were anesthetized with MS-222 (Tricaine methane sulfonate 200 mg/L river water) at the site of capture. A small 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. In addition to the temporal samples collected from the Rio Grande, samples (fin clips) were also included from 50 different captive stocks sampled between 2000 and 2012.

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) and *Ca6* and *Ca8* (Dimsoski *et al*. 2000) and *Ppro118* and *Ppro126* (Bessert & Orti 2003). The following pairs of loci were amplified using multiplex PCR: *Lco1/ Ca6* and *Lco6*/ *Lco7* (1X PCR buffer, 3 mM $MgCl_2$, 125 µM deoxyribonucleotide triphosphates [dNTPs], 0.40-0.50 micromol [µM] each primer, 0.375 units TAQ [*Thermus aquaticus*] 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: one denaturation cycle of 92°C for 2 mins followed by 30 cycles of 90 °C for 20s, 50°C for 20 s, 72°C for 30s. For *Ppro 118*/*Ppro126* cycling conditions were one denaturation cycle of 92°C for 2 mins followed by 30 cycles of 90 °C for 20s, 60°C for 20 s, 72°C for 30s. Samples that appeared homozygous at locus *Ppro118* were amplified again to check allele designations. Primer concentrations in multiplex reactions were varied to facilitate equal amplification of both loci. Prior to electrophoresis 1.2µl of PCR product was mixed with 1.2µl of a solution comprised of formamide (62.5%), ABI ROX400 size standard (12.5%) and loading buffer (25%) and denatured at 93 °C for 2 minutes. The following microsatellite PCR products for loci *Lco3, Lco6, Lco7,* and *Ca6* were run on an ABI 377 automated DNA sequencer at 50°C for 2.5 hours. *Ppro 118*/*Ppro126, Lco1, Lco8*and *Ca8* PCR products were run on an ABI 3100 automated capillary sequencer. One microliter of PCR product was mixed with 10µl of formamide and

0.3µl of HD400 size standard and denatured at 93°C for 5 minutes prior to loading. Genotype data were obtained using 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 MgCl2, 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 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. Genbank Accession numbers are provided in Alò and Turner (2005) and Moyer et al. (2005). Three additional haplotypes were identified (Genbank Accession numbers: JN543958-JN543960).

Statistical Analysis

Microsatellite data were checked for errors using MICROSATELLITE TOOLKIT (add-in for Microsoft Excel, written by S. Park, available at http://animalgenomics.ucd.ie/sdepark/ms-toolkit/. Genepop (Raymond and Rousett 1995) was used to assess whether there were significant departures from Hardy-Weinberg equilibrium (HWE) using the procedure of Guo and Thompson (1992). Global tests for linkage disequilibrium were conducted for all pairs of loci in each collection, using FSTAT vers. 2.9.3.1 (Goudet 1995). 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 co-efficients (F_{IS}) were obtained using FSTAT. Estimates of unbiased gene diversity (h) were obtained using Arlequin vers. 3.11 (Excoffier et al. 2005) for mitochondrial DNA data. Haplotype richness and gene diversity (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.

In some cases sample sizes differed between collections particularly between some samples collected early in the study and those collected more recently. As the number of alleles and expected heterozygosity are dependent on sample size, we used resampling to examine the effect of sample size on diversity measures. For microsatellites, 1000 random subsamples (n=43 in 1987) were drawn without replacement from each temporal sample. Diversity and 95% CIs were calculated for each locus across subsamples and a mean was obtained across loci for each statistics (corrected number of alleles [N_{ac}], gene diversity [H_{ec}], heterozygosity [H_{oc}]). This analysis was conducted in the R statistical package (www.r-project.org). To facilitate comparisons among collections obtained from different river reaches across years, 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 vers. 3.11 (Excoffier et al. 2005). Hierarchical analysis of molecular variance (AMOVA) was conducted to test whether a significant proportion of genetic variance was partitioned into components attributable to differences among wild, captive-spawned, and captive-reared stocks [i.e. wild-caught eggs 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 changes in microsatellite allele frequencies across annual samples, using the temporal method (Nei and Tajima 1981; Waples 1989) implemented in NeEstimator (Peel et al. 2004) and a pseudo-maximum likelihood procedure implemented in MLNE version 2.3 (Wang 2001). 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 TempoFs (www.zoologi.su.se/_ryman), was used to estimate N_{eV}. Rio Grande silvery minnows were sampled under Plan I (prior to reproduction, with replacement) for all methods; therefore, calculations of N_{eV} via TempoFs 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 in TempoFs 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 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}, we used the linkage disequilibrium method (Hill 1981) to estimate N_{eD} from microsatellite DNA data for each annual sample (including wild, captive-spawned and wild-caught eggs), using the program LDNE (Waples and Do 2008) and methods described in Osborne et al. (2010). Single sample N_e methods (such as those provided by LDNE) 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).

Results for 2012

Microsatellites- Genetic Diversity

In 2012, 517 Rio Grande silvery minnow were collected from the Angostura, Isleta and San Acacia reaches of the middle Rio Grande. A total of 7074 fish have been genotyped for nine microsatellite loci over the 14-year study (this includes samples released at Big Bend). Microsatellite locus *Ca6* was the least variable with 10 alleles detected across all populations whereas *Ppro118* was the most variable with 63 alleles. After sequential Bonferroni correction for multiple comparisons there were 233 departures from Hardy-Weinberg equilibrium (HWE) among 756 comparisons. Four loci (*Lco3, Lco6, Ca6, Ppro126*) conformed to HWE in all or nearly all comparisons. Micro-Checker suggested that null alleles probably caused departures from HWE. Across 2341 comparisons there were 40 instances of linkage disequilibrium among loci with one exception (2010) these involved captive lots. In 2012, measures of diversity calculated from microsatellite data were practically identical to those recorded in 2011 (Table 3, Figure 1). Average number of alleles (from resampling to account for differences in sample size between collections) plateaued in the Angostura reach from 2007 to 2012 (with the exception of 2011). This pattern was not seen in the collections from the Isleta or San Acacia reaches. In the Isleta reach, the average number of alleles continued an upward trend whilst in the San Acacia reach number of alleles increased after declines seen between 2007 and 2011 (Figure 2).

Mt-DNA- Genetic Diversity Across the 15-year time series, 17 mtDNA haplotypes were identified among 7074 individuals assayed (Table 4). Differentiation among haplotypes was low, with one to six substitutions among them. Haplotype A was the most common in all samples except Cs-An-02 (captive spawned) which was monomorphic for haplotype D. Three haplotypes (C, D, F) were present at moderate frequencies (>5%) and 11 haplotypes were considered rare (present at frequencies < 5%). Across the entire time series, haplotype diversity was highest in the 1987 sample (h=0.743) and lowest in 2000 (h=0.364) (Table 4). In 2012, haplotype diversity

was 0.658 and 11 haplotypes were detected. In 2012, haplotype diversity (h=0.693) and number of haplotypes (11) were highest for the fishes collected from the San Acacia reach. Haplotype diversity was lowest in the Angostura reach (0.594) (Figure 3).

Microsatellites- Population Structure Hierarchical analysis of molecular variance was conducted by grouping temporal samples by river reach. Values were not significantly different from zero, indicating that river reach did not explain a significant portion of genetic variance (F_{CT}=-0.00001, P= 0.7155); a result consistent with previous years.

Mt-DNA- Population Structure

Φ-statistics were calculated between wild samples collected in 1987 and from 1999-2010 partitioned by river reach. Results indicated that genetic differences among river reaches (Angostura, Isleta and San Acacia) (Φ_{CT} = -0.0008, P = 0.940) were not significantly different from zero.

Genetic Effective Size

For 2011-2012 estimates of variance effective size calculated using three methods (across nine loci) were 462 (moments, 95% CIs 286,855), infinity (TempoFs, 95% CIs 9829, infinity) and 803 (MLNE, 95% CIs 527, 1523) (Figure 4). All estimates increased over the previous temporal comparison (2010-2011). Estimates of female effective size were infinity (95% CIs 161-infinity) (moments-based) and N_{ef}=43,745 (MLNE, 95% CIs 210 to infinity) (Figure 5). There has been a trend of increasing variance effective size in both the Isleta and San Acacia reaches since lows recorded in 2006-2007. The trend of effective size in the Angostura reach has been stable at value of around 200 for all comparisons beginning with 2008-2009. Effective size was also estimated using the linkage disequilibrium method. For the wild population in 2012, N_{eD} was 10,064 (Figure 6) and from 27 to 121 for captive stocks released in the middle Rio Grande (Table 3).

Big Bend

For fish released at Big Bend measures of microsatellite diversity (H_{ec}, H_{oc} and N_{ac}) were similar to that of the wild New Mexico population. There were ten mtDNA haplotypes represented in the fish released in fall 2011 and gene diversity of individual lots was comparable to the wild population (NM) ranging from 0.628-0.716. Pairwise Φ-statistics indicated that genetic differences among the groups of fish released at each of four localities were not significantly different from zero. For captive-bred fish released at Big Bend in fall of 2011, N_{eD} ranged from 110 to 1529. Estimates of N_{eD} were substantially higher (277-2425) for the three lots of fish that were wild-caught eggs reared in captivity (Table 3).

Discussion

Genetic status of the species in 2012

Extensive demographic surveys show that the wild population of Rio Grande silvery minnow has experienced multiple, order of magnitude changes in density over the past two decades (U.S. Fish and Wildlife Service 2010). From 2000-2004 densities of Rio Grande silvery minnow were less than one fish per 100 m^2 and during this time the threat of extinction in the wild was acute. For both microsatellites and mtDNA there is considerable inter-annual variability in gene diversity metrics and effective size estimates from 1987 and 1999-2004. Following commencement of population supplementation with fish reared in captivity, there has been a general trend was toward stabilization and marginal increases in mtDNA and microsatellite diversity and the number of alleles/haplotypes. Inter-annual variability in all of these measures decreased after 2005 with this trend continuing in 2012. Microsatellite gene diversity and heterozygosity was very similar to values recorded in previous years. Allelic richness has remained stable since 2006 and continued to do so in 2012. Mitochondrial haplotype diversity was the highest recorded since 1987 whilst haplotype richness similar to 2011.

Estimates (from microsatellite data) of variance effective population size for 2011-2012 calculated using the temporal method increased from values calculated for the previous time period (2010-2011). Results were consistent across the three methods used to calculate variance effective size. The trend of increasing effective size is encouraging and suggests that there is less change in allele frequencies from year to year; likely attributable to augmentation program. Despite the increase in genetic effective size it is still a fraction of the estimated census size of the population. Low N_{eV} results from an important interaction of life history (e.g., pelagic eggs and larvae) and habitat fragmentation by dams that results in high variance in reproductive success among spawning pairs in the Rio Grande (Alò & Turner 2005, Osborne *et al*. 2005, Turner *et al*. 2006). It is important to note that the negative interaction of life history and fragmentation occurs even when recruitment is strong because downstream displacement of eggs and larvae is arguably expected to be greater when spring flows are higher (Dudley 2004). Estimates of effective size (moments) made from mitochondrial DNA haplotype frequency data also increased for the 2011-2012 time period and were indistinguishable from infinity.

We also used the linkage disequilibrium method to estimate effective size. 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} (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_{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 and Luikart 2007); therefore, N_{eI} 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}.

Reach Specific Findings

In 2008, US FWS ceased supporting the population of Rio Grande silvery minnow in the Angostura reach with captive fish to test the efficacy of stocking (J. Remshardt pers. comm.). This trial was to run for a period of 5 years unless catch rates fell below a predetermined level (which occurred in 2012). Population augmentation continued in the Isleta and San Acacia reaches during this period. Due to downstream displacement of eggs and larvae and the preclusion of upstream movement of fishes (due to the presence of diversion dams) that could offset the downstream loss, we predicted that genetic diversity would gradually decrease in the Angostura reach in the absence of stocking. We also might predict a gradual increase in inbreeding in this reach if numbers of fish declined to a sufficiently small number. When stocking in the Angostura reach commenced in 2002 we saw an increase in diversity which is not surprising as there were very few fish remaining here in the late 1990s-early 2000's. Since 2008, the average number of alleles has remained stable and observed heterozygosity has increased. However, for mtDNA there has been an overall decrease in haplotype richness. In contrast since 2008, average number of alleles/haplotypes and haplotype diversity have increased in the Isleta reach. Patterns in the San Acacia reach are less discernible as there is considerable inter-annual variability in all measures of diversity likely due to changes in population size associated with the extent of river drying in this reach.

In 2012, we also estimated variance effective size on a reach by reach basis (not all temporal comparisons were possible because of small sample sizes obtained from some reaches in some years). Since 2005, we have seen a distinct positive trend in N_{eV} in both the Isleta and San Acacia reaches, whilst for the Angostura reach there was an increase for consecutive pairwise comparisons from 2006-2007 to 2008-2009, N_{eV} has plateaued each year since. We attribute these findings to variance in reproductive success in the Angostura reach. Whilst this occurs amongst individuals spawning in the Isleta and San Acacia reaches, supplementation of these populations with fish bred and/or reared in captivity (which reduces variance in reproductive success amongst individuals) helps to reduces changes in allele frequencies from year to year (Osborne et al. 2012). Interestingly, there is one temporal comparison (2005-2006) where N_{eV} is very similar between reaches and this corresponds to the year (2005) in which the highest catch per unit effort and high spring runoff were recorded.

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

In fall 2011, three stocks released in the middle Rio Grande New Mexico was derived primarily from wild-caught eggs and wild-caught young of year. The remaining fish released were derived from captive spawning. The stocks reared from wild-caught eggs had higher average number of alleles/haplotypes and higher genetic effective size (estimated using the linkage disequilibrium method) than the captive spawned stocks. 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.

Genetic diversity of captive stocks released to Big Bend National Park, Texas

In 2012 we characterized genetic variation in four lots of captive spawned fish that were released at four localities in the Big Bend National Park in the fall of 2011. These fish were bred at Dexter National Fish Hatchery and Technology Center. Diversity measures for these stocks were comparable to the wild population. This baseline data will allow us to track the genetic fate of the reintroduced population. Having a genetically diverse population initially, will help to reduce the chances of a genetic bottleneck and hence to maximize the long-term viability of this population. We also estimated the effective number of breeders for these captive lots released to Big Bend and in all cases values of N_{eD} were moderate to high (110-1529).

Conclusions

Fifteen 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 been maintained over the duration of the study. Variance effective size remained lower than inbreeding effective size and substantially lower than population density, suggesting that the interaction of early life history and river fragmentation is still exerting downward pressure on this metric, despite supplementation. In the absence of supplementation, we predict convergence of inbreeding and variance effective sizes and substantive losses of genetic diversity each generation thereafter. Analysis of reach specific data can provide a glimpse into the likely genetic effects of not supplementing the wild population without first addressing the underlying causes of population decline. In only four years of not stocking in the Angostura reach we saw some measures of diversity (including those most sensitive to population decline) plateau including genetic effective size. The same clear trend was not apparent in the Isleta or San Acacia reaches. This is further evidence that diversity in the population is being largely maintained by release of captive reared fish. These results also highlight 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. This is especially likely in the event of a catastrophic decline of the wild population, followed by heavy stocking with captive bred fish which may be less well suited to the demands life in the wild.

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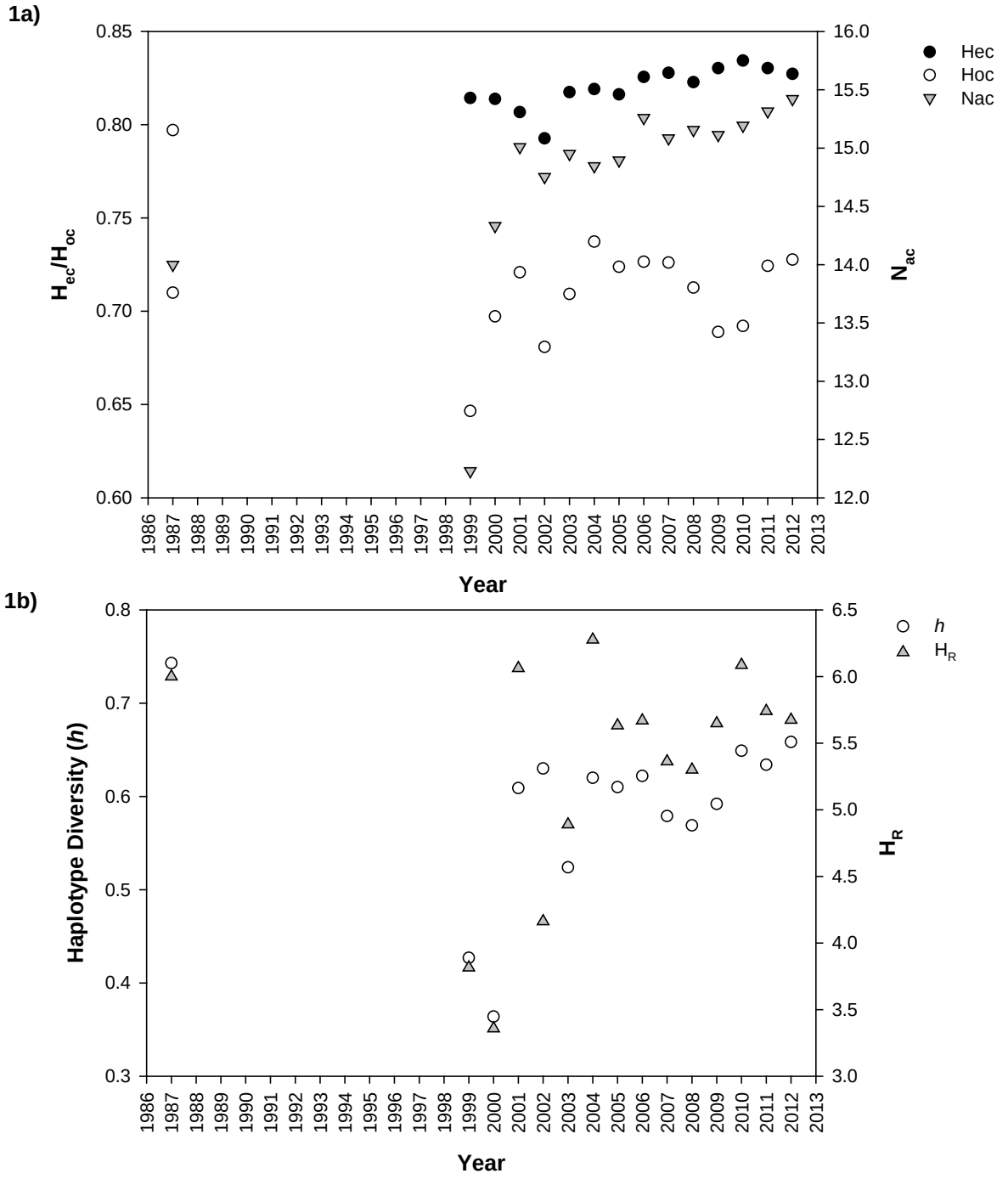

Figure 1. (**a**) Diversity metrics from microsatellite data obatined using resampling (H_{ec}, H_{oc}, N_{ac}) and (**b**) haplotype diversity (*h*) and haplotype richness (H_R) from mtDNA-ND4 by year.

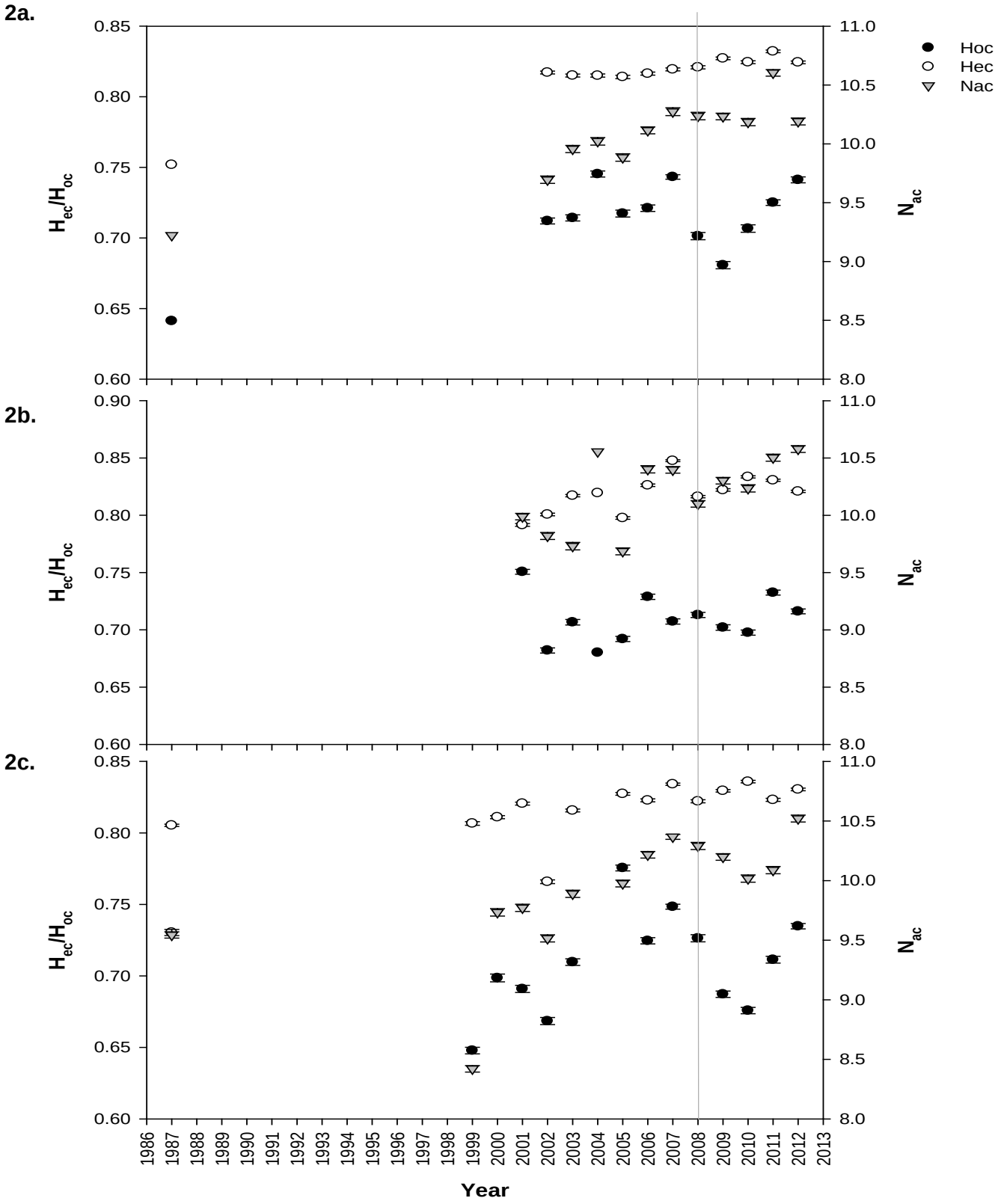

Figure 2. Diversity metrics from microsatellite data obatined using resampling (H_{ec}, H_{oc}, N_{ac}) by year and river reach (**a**) Angostura, (**b**) Isleta and (**c**) San Acacia. Dashed line delimites commencement of not stocking the Angostura reach (2008-2012).

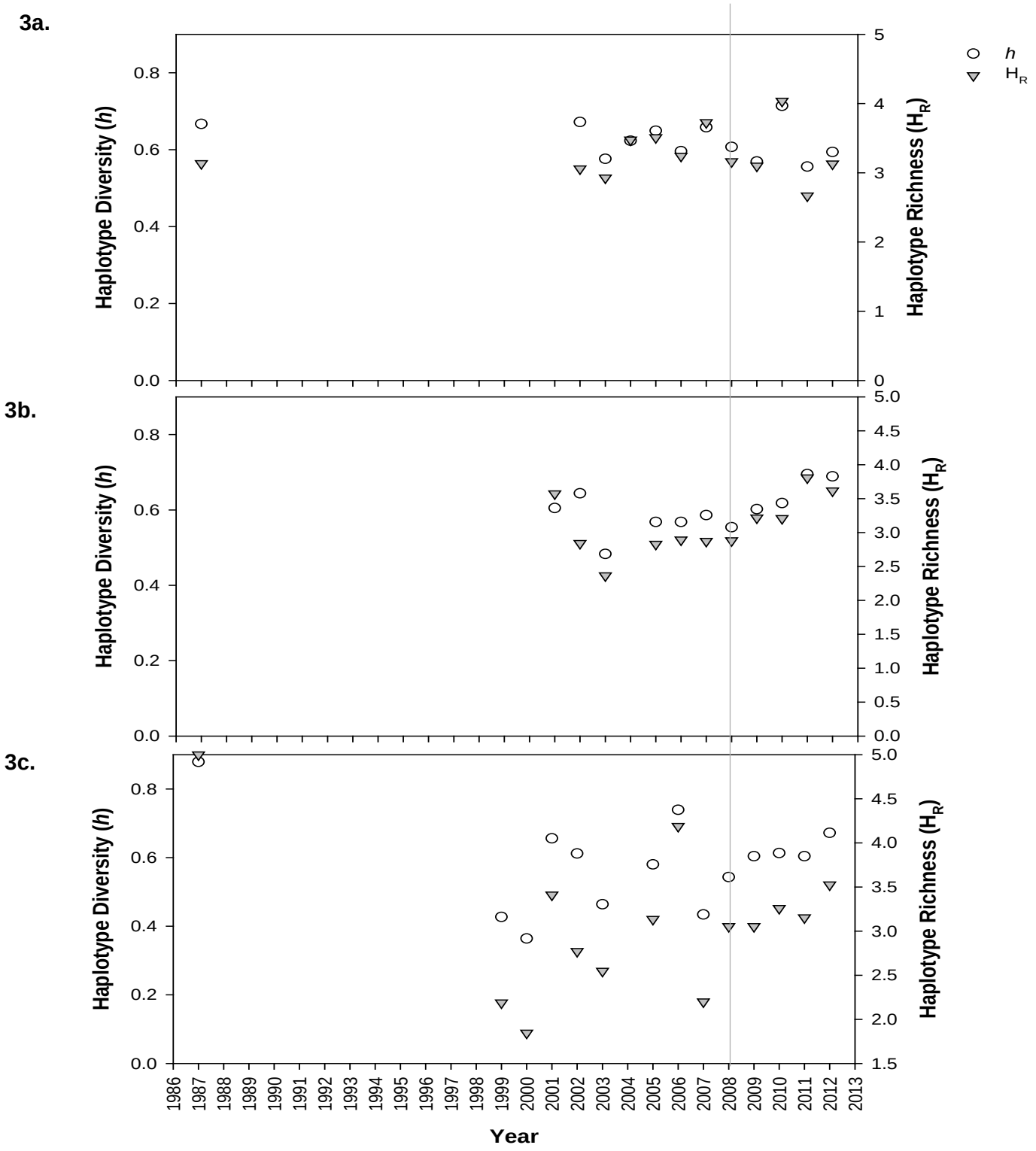

Figure 3. (**a**) Haplotype diversity (*h*)and haplotype richness (H_R) from mtDNA-ND4 by year and river reach (**a**) Angostura, (**b**) Isleta and (**c**) San Acacia. Grey line delimites temporary cessation of stocking the Angostura reach (2008-2012).

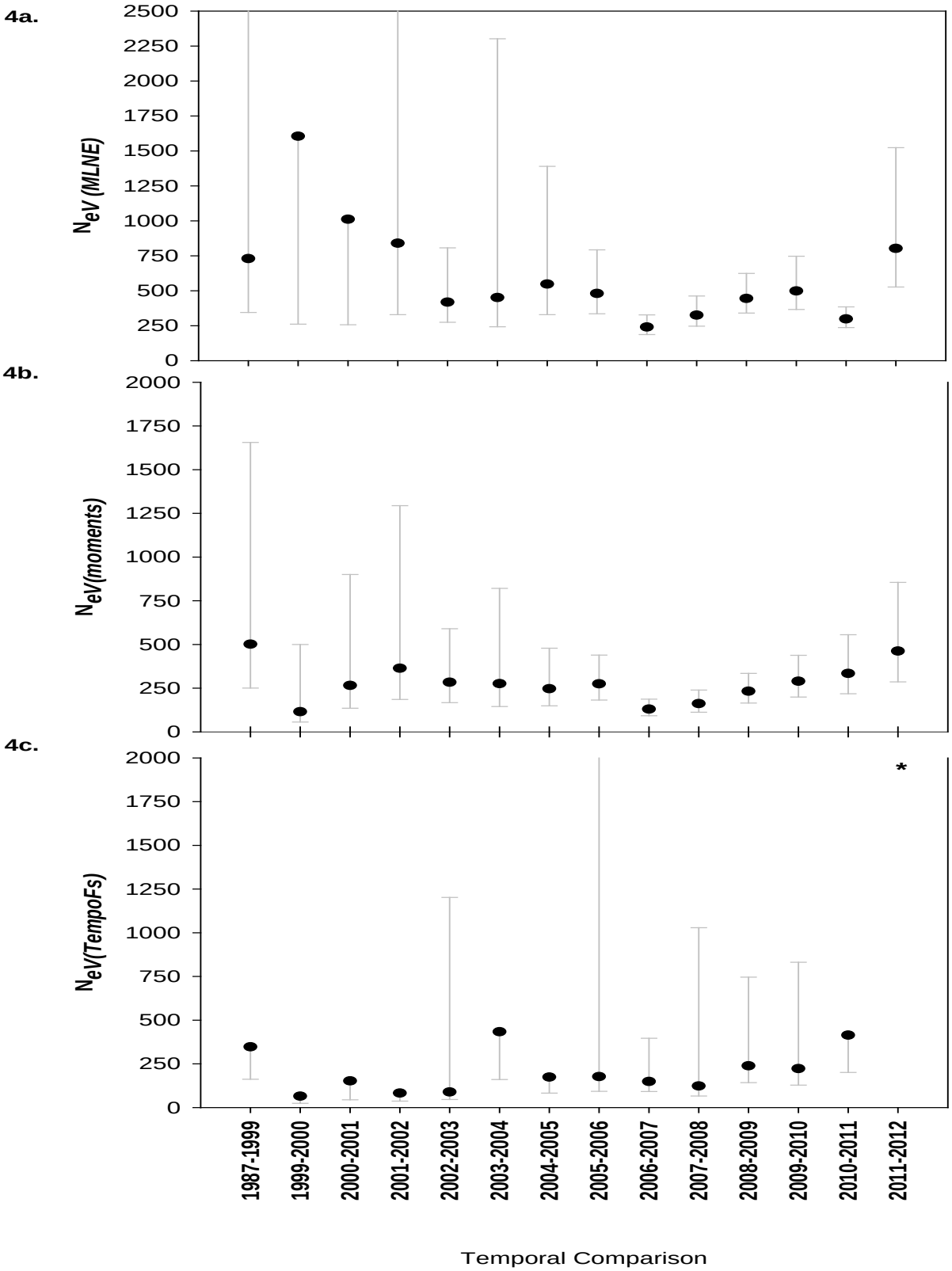

Figure 4. Variance effective size calculated from microsatellite data using (**a**) MLNE, (**b**) moments-based and (**c**) TempoFs methods and associated 95% CIs (absence of +95% error bars indicates upper bounds of infinity). Estimates of infinity are incated by an asterisks.

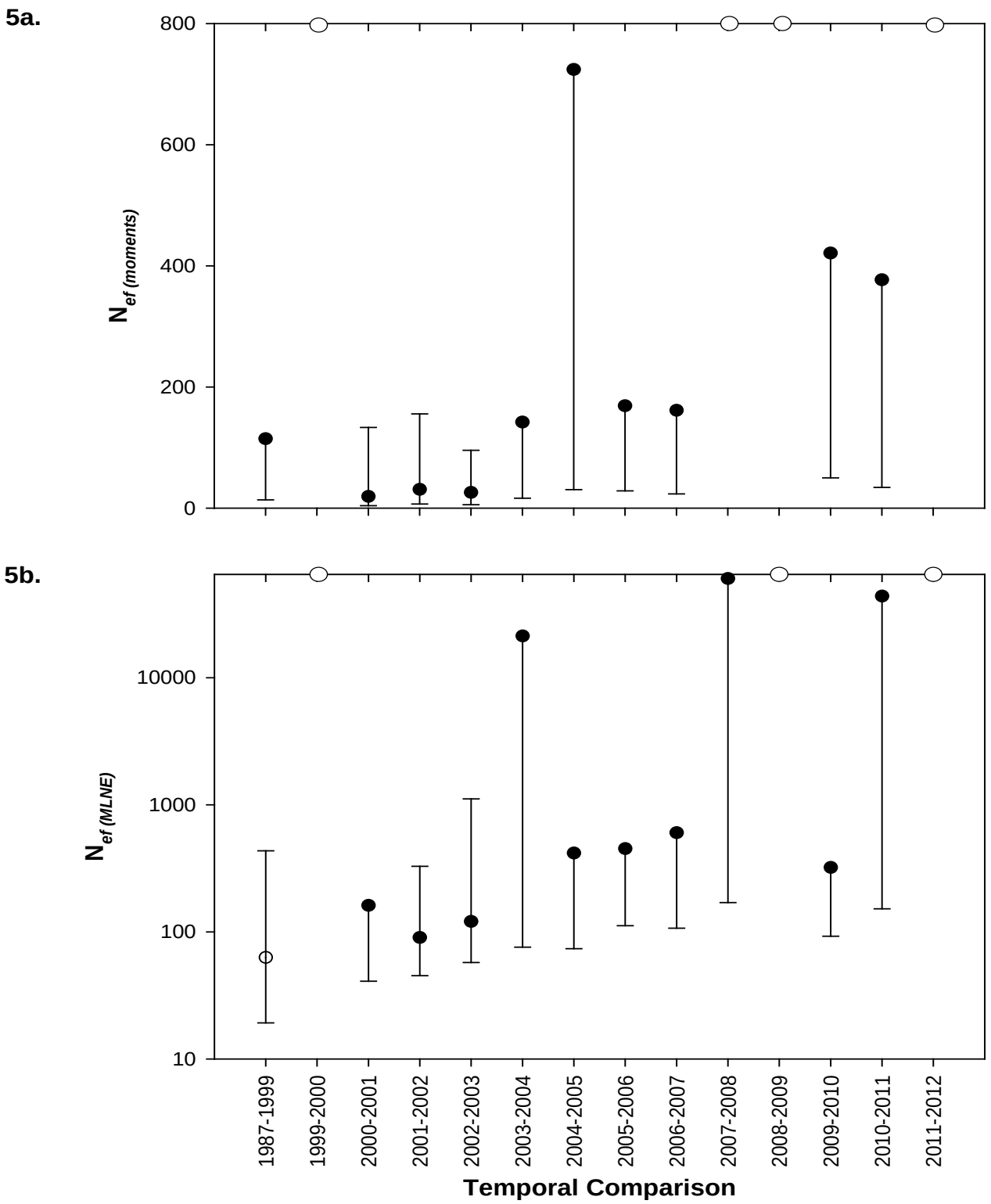

Figure 5. Variance effective size calculated from mtDNA-ND4 data using (**a**) moments-based and (**b**) MLNE and associated 95% CIs (absence of +95% error bars indicates upper bounds of infinity). Estimates of infinity are incated by an open circle.

6.

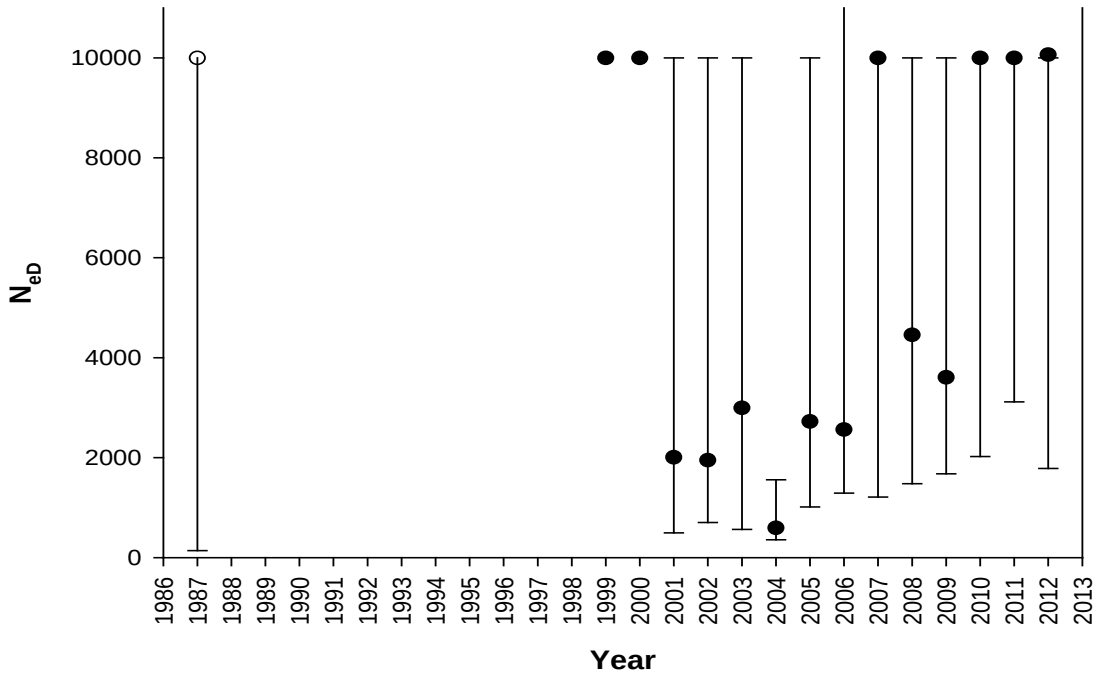

Figure 6. Estimates of genetic effective size (N_{eD}) and 95% confidence intervals estimated using the linkage disequilibrium across the temporal time series.

7.

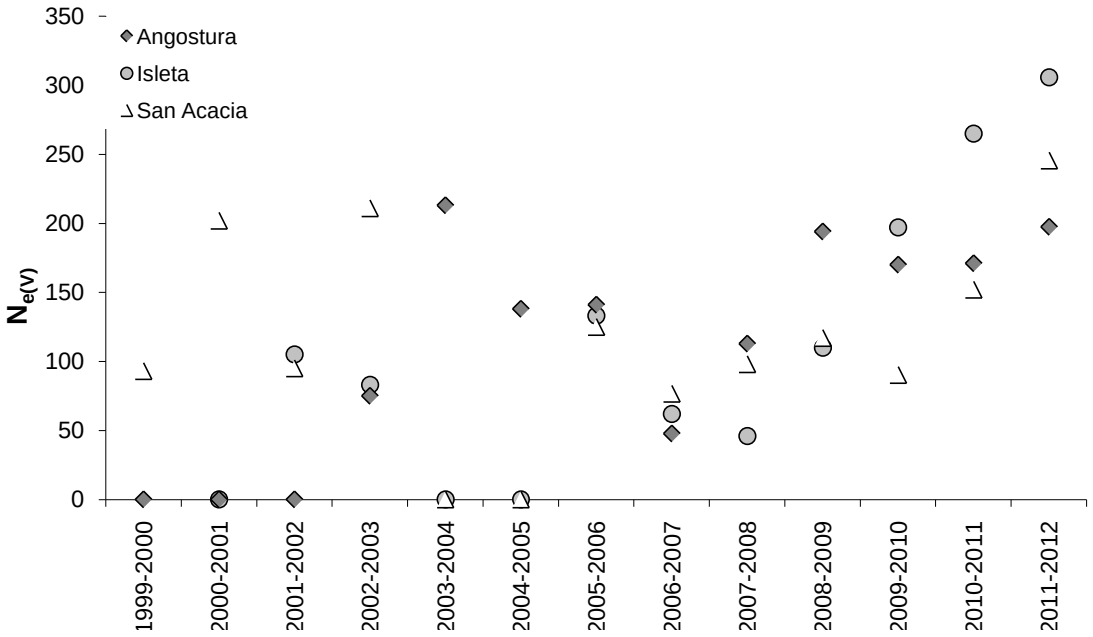

Figure 7. Variance effective size calculated from microsatellite data using the moments-based method by river reach. Error bars are not shown for clarity. Points on the zero line indicate samples for which no samples were available.

Table 3. Diversity statistics for microasatellites obatined using resampling (N=sample size, N_{ac}=average number of alleles across loci, H_{ec}=Nei's gene diversity, H_{oc}=observed heterozygosity) and average inbreeding co-efficient and diversity statistics from mtDNA data: *h* = haplotype diversity, H_R=haplotype richness. Linkage disequilibrium estimates of effective size are also given

			Microsatellites							***MtDNA-ND4***		
Wild-MRG	**N**	**N_{ac}**	**H_{ec}**	**H_{oc}**	**F_{IS}**	**N_{eD}**	**-95%**	**95%**	**N**	**h**	**H_R**	**N haps**
1987	43	14.000	0.797	0.710	0.111	∞	139.3	∞	37	0.743	6	7
1999	46	12.229	0.814	0.647	0.21	∞	∞	∞	44	0.427	3.816	5
2000	194	14.332	0.814	0.697	0.145	∞	∞	∞	124	0.364	3.359	6
2001	128	15.008	0.807	0.721	0.107	2007.7	495.1	∞	122	0.609	6.063	10
2002	389	14.752	0.793	0.681	0.143	1950.6	701.7	∞	387	0.63	4.163	8
2003	169	14.951	0.817	0.709	0.134	2997.7	563.8	∞	167	0.524	4.89	9
2004	162	14.845	0.819	0.737	0.1	595.5	357.2	1558.7	161	0.62	6.277	10
2005	394	14.895	0.816	0.724	0.113	2724.3	1013.5	∞	396	0.61	5.633	10
2006	383	15.259	0.826	0.727	0.122	2561.7	1291.4	34063.9	378	0.622	5.67	10
2007	218	15.084	0.828	0.726	0.123	∞	1210.7	∞	218	0.579	5.363	10
2008	474	15.156	0.823	0.713	0.135	4458.5	1478.5	∞	466	0.569	5.301	11
2009	476	15.113	0.830	0.689	0.172	3607.6	1676.9	∞	472	0.592	5.649	12
2010	440	15.192	0.834	0.692	0.172	∞	2022.8	∞	433	0.649	6.087	9
2011	362	15.315	0.830	0.724	0.13	∞	3116.6	∞	359	0.634	5.741	11
2012	517	15.421	0.827	0.728	0.123	10064.2	1782.1	∞	522	0.6583	5.675	11
Wild-caught eggs												
WcE01*	178	14.756	0.819	0.651	0.206	1379.6	655.6	∞	157	0.627	6.999	8
WC_SA_01	50	13.947	0.830	0.727	0.070	85.6	54.1	173.4	51	0.624	6	6
WC_AN_02	50	12.118	0.784	0.731	0.126	∞	238.3	∞	49	0.481	2.949	3
Wc_SA_02	81	14.946	0.818	0.680	0.171	∞	461.7	∞	80	0.702	7.376	8
WCE-SA-03	51	14.985	0.830	0.696	0.164	5008.5	307.6	∞	51	0.714	7.848	8
MJO07_005	54	15.310	0.827	0.738	0.091	60.4	48.3	78.5	53	0.602	6.733	7
MJO07_006	49	15.637	0.814	0.723	0.108	1065.0	195.9	∞	48	0.581	5.962	6
MJO11_015	49	15.420	0.818	0.694	0.154	871.3	269.9	∞	49	0.625	5.396	7
MJO11_016	50	15.288	0.837	0.756	0.097	2424.6	358.5	∞	50	0.5984	5.791	7
MJO11_017	50	14.488	0.813	0.720	0.115	277.2	142.8	2069.9	46	0.7575	6.568	8
Captive-spawned												
MJO06_29	50	11.371	0.804	0.745	0.074	42.2	28.7	68.7	50	0.517	5	5
Cs_01	64	12.807	0.794	0.658	0.172	43.7	35.6	55	58	0.46	4.982	5
Cs_AN_02	51	8.476	0.685	0.675	0.015	21.6	14.9	32.5	51	0	1	1
CS_SA_02	53	13.145	0.802	0.673	0.163	72.7	52.5	110.9	53	0.751	5.919	6
TFT039	51	12.766	0.806	0.700	0.133	106.3	56.0	433.5	52	0.558	3.995	4
Cs04	50	14.089	0.823	0.690	0.163	65.5	45.7	105.7	47	0.586	5.911	6
TFT04_23	50	11.652	0.779	0.683	0.124	20.4	16.5	25.4	47	0.593	4.996	5

Captive-spawned	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	h	H_R	N haps
TFT04_24	48	11.758	0.828	0.717	0.135	40.2	29.7	57.8	48	0.609	4.949	5
TFT04_25	50	11.656	0.810	0.768	0.053	24.9	20.0	31.5	53	0.702	5.934	6
TFT04_29	54	14.010	0.839	0.762	0.092	-423.6	532.0	∞	53	0.609	4.903	5
TFT04_30	56	14.700	0.825	0.727	0.121	323.1	134.0	∞	45	0.656	4.79	5
TFT04_31	50	12.797	0.805	0.701	0.130	83.2	54.7	154.7	50	0.706	6.865	7
TFT05_006	50	10.306	0.792	0.649	0.183	49.4	38.8	65.7	50	0.625	5.803	6
TFT05_007	49	12.151	0.797	0.704	0.117	86.6	53.2	191.3	48	0.55	4.884	5
TFT05_008	50	11.146	0.804	0.663	0.178	32.2	26.7	39.5	49	0.611	4.934	5
TFT05_009	50	12.903	0.804	0.717	0.109	219.9	98.8	∞	50	0.506	3.996	4
TFT05_011	51	12.560	0.808	0.693	0.144	136.6	81.0	354	53	0.573	5.853	6
MJO06_25	50	14.845	0.813	0.721	0.115	184.5	110.1	487.9	49	0.635	4.934	5
MJO06_28	50	12.407	0.805	0.705	0.125	87.6	57.2	164.3	50	0.738	4.996	5
MJO07_007	50	13.162	0.813	0.739	0.114	∞	520.6	∞	50	0.605	4.869	5
LL_11	50	14.183	0.829	0.738	0.110	301.5	123.0	∞	49	0.681	0.37	5
MJO10_005	49	14.035	0.839	0.700	0.167	259.7	86.9	∞	44	0.7104	2.999	6
MJO10_006	49	12.360	0.782	0.698	0.108	58.8	32.3	163.4	49	0.664	4.875	6
MJO10_007	48	14.059	0.825	0.742	0.101	106.1	60.2	312.1	48	0.518	5.48	7
MJO11_005	48	13.966	0.810	0.730	0.100	118.2	81.6	201.2	47	0.594	2.999	4
MJO11_011	50	11.872	0.769	0.693	0.101	36.7	30.3	45.4	51	0.6871	6.733	8
MJO11_012	50	11.606	0.785	0.712	0.094	26.8	21.1	34.8	50	0.5624	3.922	5
MJO11_013	48	13.354	0.806	0.715	0.115	46.4	34.0	67.9	48	0.3369	3.703	5
MJO11_014	50	13.771	0.829	0.754	0.092	68.4	51.7	96.7	50	0.4694	4.598	6
LL_12	49	12.475	0.794	0.684	0.141	40.9	33.0	52	48	0.6259	4.493	6
MJO12_009	50	14.026	0.829	0.721	0.133	61.5	45.9	88.3	49	0.6012	2.998	4
MJO12_010	50	14.163	0.810	0.719	0.113	121.0	68.6	371	50	0.6424	5.706	7
Big Bend												
MJO08-006	50	14.761	0.826	0.669	0.192	304.3	173.4	1047.5	47	0.662	6.911	7
MJO08-007	50	14.427	0.841	0.721	0.144	392.8	160.8	∞	50	0.625	6.803	7
MJO08-008	50	15.019	0.835	0.711	0.149	614.7	194.9	∞	49	0.706	5.997	6
MJO08-009	51	15.315	0.843	0.715	0.153	174.6	106.1	425.8	51	0.658	5.995	6
MJO09-001	68	15.092	0.818	0.706	0.138	217.6	134.7	487.1	67	0.612	8.326	9
MJO09-002	72	14.823	0.797	0.670	0.162	642.9	237.8	∞	72	0.555	7.189	8
MJO09-003	71	14.624	0.811	0.719	0.115	257.8	131.5	1736.7	71	0.64	5.76	6
MJO09-004	69	14.603	0.817	0.715	0.128	425.1	186.2	∞	66	0.442	4.768	6
MJO09-005	50	15.351	0.827	0.690	0.166	430.6	217.4	5708.1	49	0.735	5	5
MJO09-006	50	14.413	0.821	0.706	0.141	109.7	76.4	182.3	50	0.53	4	4
MJO09-007	50	14.779	0.823	0.703	0.146	207.9	135.3	418.9	50	0.675	5.68	6
MJO09-008	50	15.121	0.819	0.712	0.133	176.3	101.1	537.4	50	0.776	6.803	7
MJO09-009	50	14.933	0.820	0.697	0.151	187.2	112.9	472.6	50	0.504	7.799	8
MJO09-010	48	13.917	0.816	0.697	0.146	408.8	177.9	∞	48	0.681	8.928	9
MJO09-011	50	12.361	0.792	0.657	0.173	78.8	59.2	112.6	50	0.767	5	5

Big Bend	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	h	H_R	N haps
MJO09-012	49	12.298	0.803	0.675	0.161	43.3	33.3	59	43	0.666	7	7
MJO09-013	50	13.271	0.811	0.670	0.176	1032.0	33.3	∞	50	0.563	7.792	8
MJO09-014	50	14.048	0.803	0.682	0.154	122.0	86.9	195.2	50	0.742	7.995	8
MJO10_001	50	14.302	0.841	0.743	0.111	997.6	226.5	∞	51	0.63	5.83	8
MJO10_002	50	15.310	0.831	0.759	0.094	914.1	182.8	∞	49	0.659	3.932	5
MJO10_003	48	15.133	0.821	0.745	0.094	∞	409.1	∞	48	0.504	4.312	6
MJO10_004	50	15.444	0.804	0.767	0.046	496.9	189.7	∞	49	0.639	3.51	5
MJO11-007	50	14.406	0.822	0.693	0.158	675.0	215.0	∞	50	0.6694	3.725	5
MJO11-008	50	14.682	0.825	0.697	0.157	1529.3	278.8	∞	50	0.6278	5.401	7
MJO11-009	50	13.798	0.797	0.650	0.187	110.0	84.2	154.4	51	0.6431	6.236	8
MJO11-010	50	14.556	0.821	0.732	0.109	256.3	129.2	2577.3	47	0.716	7.099	9

Table 4. MtDNA haplotype frequencies for the wild middle Rio Grande population, fish reared from wild-caught, fish reared from cative spawning and for fish released at Big Bend.

	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T	W	V
Wild																	
1987	0.459	0.162	0.162	0.054	0.081	0.027	-	-	0.054	-	-	-	-	-	-	-	-
1999	0.75	-	0.114	0.068	0.045	0.023	-	-	-	-	-	-	-	-	-	-	-
2000	0.79	0.008	0.048	0.048	0.097	0.008	-	-	-	-	-	-	-	-	-	-	-
2001	0.607	0.09	0.057	0.033	0.098	0.074	0.008	0.016	0.008	-	-	0.008	-	-	-	-	-
2002	0.556	0.199	0.137	0.01	0.059	0.034	-	0.003	-	-	-	0.003	-	-	-	-	-
2003	0.671	0.054	0.15	0.03	0.054	0.012	-	0.006	0.006	-	-	0.018	-	-	-	-	-
2004	0.596	0.087	0.106	0.019	0.075	0.05	0.012	-	0.019	-	0.006	0.031	-	-	-	-	-
2005	0.598	0.126	0.088	0.028	0.086	0.018	0.015	0.003	0.028	-	-	0.01	-	-	-	-	-
2006	0.587	0.135	0.093	0.048	0.048	0.048	0.003	-	0.029	-	-	0.008	-	-	0.003	-	-
2007	0.628	0.11	0.083	0.023	0.087	0.037	0.005	-	0.005	-	-	0.018	0.005	-	-	-	-
2008	0.635	0.12	0.079	0.026	0.067	0.045	0.004	-	0.009	-	0.002	0.006	-	0.006	-	-	-
2009	0.614	0.14	0.076	0.028	0.064	0.034	0.006	0.004	0.019	-	0.002	0.011	-	0.002	-	-	-
2010	0.562	0.124	0.097	0.032	0.069	0.053	0.014	-	0.016	-	-	0.032	-	-	-	-	-
2011	0.574	0.142	0.109	0.028	0.064	0.031	0.006	-	0.031	-	-	0.011	0.003	0.003	-	-	-
2012	0.538	0.165	0.116	0.034	0.072	0.030	0.004	0.004	0.017	-	-	0.017	0.002	-	-	-	-
Wild-caught eggs																	
WcE-01	0.573	0.197	0.051	0.064	0.064	0.032	-	-	0.013	0.006	-	-	-	-	-	-	-
WcE-SA-01	0.569	0.137	0.059	0.059	0.098	0.078	-	-	-	-	-	-	-	-	-	-	-
WcE-An-02	0.653	0.02	0.327	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WcE-SA02	0.488	0.225	0.05	0.013	0.138	0.05	-	-	0.038	-	-	-	-	-	-	-	-
WcE-SA-03	0.49	0.078	0.196	0.059	0.098	0.039	-	-	0.02	-	-	0.02	-	-	-	-	-
MJO07-005	0.604	0.094	0.019	0.019	0.17	0.075	-	0.019	-	-	-	-	-	-	-	-	-
MJO07-006	0.604	0.083	0.125	0.021	0.083	0.042	-	-	-	-	-	0.042	-	-	-	-	-
MJO11_15	0.571	0.224	0.041	0.020	0.041	0.082	-	-	0.020	-	-	-	-	-	-	-	-
MJO11_16	0.620	0.120	0.060	-	0.080	0.040	-	-	0.040	-	-	0.040	-	-	-	-	-
MJO11_17	0.435	0.196	0.065	0.043	0.130	0.087	-	-	0.022	-	-	-	0.022	-	-	-	-

Captive spawned	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T	W	V
MJO06-29	0.68	0.14	0.08	-	0.06	-	-	-	0.04	-	-	-	-	-	-	-	-
Cs-01	0.724	0.052	-	0.034	0.069	0.121	-	-	-	-	-	-	-	-	-	-	-
Cs-An-02	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cs-SA-02	0.434	0.075	0.17	0.132	0.17	-	-	-	-	-	0.019	-	-	-	-	-	-
Cs-04	0.596	0.255	0.021	-	0.043	0.064	-	-	-	-	-	0.021	-	-	-	-	-
TFT039	0.596	0.269	0.038	-	-	0.096	-	-	-	-	-	-	-	-	-	-	-
TFT04-23	0.617	0.043	0.191	-	-	0.043	-	-	-	-	-	0.106	-	-	-	-	-
TFT04-24	0.583	0.125	0.208	-	0.021	0.063	-	-	-	-	-	-	-	-	-	-	-
TFT04-25	0.434	0.057	0.113	0.057	0.283	0.057	-	-	-	-	-	-	-	-	-	-	-
TFT04-29	0.566	0.245	-	0.075	-	0.094	-	-	0.019	-	-	-	-	-	-	-	-
TFT04-30	0.4	0.333	-	-	-	0.244	-	-	-	-	0.022	-	-	-	-	-	-
TFT04-31	0.42	0.34	0.02	-	0.06	0.04	-	-	0.1	-	-	0.02	-	-	-	-	-
TFT05-06	0.5	0.36	0.02	-	0.02	0.08	-	-	0.02	-	-	-	-	-	-	-	-
TFT05-07	0.625	0.292	0.021	0.063	-	0	-	-	-	-	-	-	-	-	-	-	-
TFT05-08	0.592	0.082	-	0.102	-	0.224	-	-	-	-	-	-	-	-	-	-	-
TFT05-09	0.68	0.16	-	-	-	0.12	-	-	0.04	-	-	-	-	-	-	-	-
TFT05-11	0.623	0.057	0.113	0.019	0.17	-	-	-	0.019	-	-	-	-	-	-	-	-
MJO06-25	0.551	0.245	0.061	-	0.061	0.082	-	-	-	-	-	-	-	-	-	-	-
MJO06-28	0.4	0.14	0.22	-	0.22	0.02	-	-	-	-	-	-	-	-	-	-	-
MJO07-007	0.56	0.02	0.12	0.28	0.02	-	-	-	-	-	-	-	-	-	-	-	-
LL_11	0.469	0.224	0.245	-	0.041	-	-	-	0.020	-	-	-	-	-	-	-	-
MJO10_005	0.477	0.182	0.159	-	0.136	0.023	-	-	-	-	-	0.023	-	-	-	-	-
MJO10_006	0.531	0.224	0.041	0.061	-	-	0.102	-	0.041	-	-	-'	-	-	-	-	-
MJO10_007	0.688	0.063	0.042	0.021	0.083	0.083	-	-	-	-	-	-	0.021	-	-	-	-
MJO11_005	0.596	0.213	0.106	-	0.085	-	-	-	-	-	-	-	-	-	-	-	-
MJO11_011	0.529	0.059	0.039	0.039	0.039	0.176	-	-	0.059	-	-	-	-	-	-	-	0.059
MJO11_012	0.640	0.120	0.040	-	-	-	-	-	0.140	-	-	0.060	-	-	-	-	-
MJO11_013	0.813	0.063	0.063	0.042	-	-	-	-	0.021	-	-	-	-	-	-	-	-
MJO11_014	0.720	0.040	0.060	0.040	-	0.120	-	-	-	-	-	0.020	-	-	-	-	-
LL_12	0.563	0.042	0.125	-	0.229	0.021	-	-	-	-	-	-	0.021	-	-	-	-
MJO12_009	0.592	0.184	0.082	-	0.143	-	-	-	-	-	-	-	-	-	-	-	-
MJO12_010	0.580	0.080	0.100	-	0.100	0.060	-	-	-	-	-	0.020	0.060	-	-	-	-

	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T	W	V
Big Bend																	
MJO08_06	0.532	0.234	0.064	0.043	0.085	0.021	-	-	-	-	-	0.021	-	-	-	-	-
MJO08_07	0.580	0.180	0.020	0.060	0.120	0.020	-	-	0.020	-	-	-	-	-	-	-	-
MJO08_08	0.490	0.204	0.061	0.082	0.122	0.041	-	-	-	-	-	-	-	-	-	-	-
MJO08_09	0.549	0.176	0.059	0.039	0.118	0.059	-	-	-	-	-	-	-	-	-	-	-
MJO09_01	0.582	0.194	0.060	0.015	0.060	0.030	-	-	0.030	-	-	0.015	-	-	-	0.015	-
MJO09_02	0.639	0.194	0.069	0.014	0.014	0.014	0.028	-	0.028	-	-	-	-	-	-	-	-
MJO09_03	0.563	0.197	0.056	-	0.127	0.014	-	-	0.042	-	-	-	-	-	-	-	-
MJO09_04	0.727	0.182	0.030	-	0.030	0.015	-	-	0.015	-	-	-	-	-	-	-	-
MJO09_05	0.449	0.163	0.163	-	0.122	0.102	-	-	-	-	-	-	-	-	-	-	-
MJO09_06	0.660	0.080	0.080	-	0.180	-	-	-	0.000	-	-	-	-	-	-	-	-
MJO09_07	0.510	0.216	0.157	-	0.078	-	-	-	0.020	-	-	0.020	-	-	-	-	-
MJO09_08	0.300	0.300	0.160	0.020	0.180	-	-	-	0.020	-	-	0.020	-	-	-	-	-
MJO09_09	0.700	0.080	0.060	0.020	0.040	0.060	-	-	0.020	-	-	-	-	0.020	-	-	-
MJO09_10	0.535	0.186	0.070	0.023	0.070	0.023	0.047	-	0.023	-	0.023	-	-	-	-	-	-
MJO09_11	0.327	0.306	0.163	-	0.082	0.122	-	-	-	-	-	-	-	-	-	-	-
MJO09_12	0.558	0.070	0.070	-	0.070	0.047	-	-	0.140	-	0.047	-	-	-	-	-	-
MJO09_13	0.640	0.040	0.040	0.020	0.180	0.040	0.020	-	0.020	-	-	-	-	-	-	-	-
MJO09_14	0.468	0.106	0.064	0.064	0.170	0.043	-	-	-	-	0.021	0.064	-	-	-	-	-
MJO10-01	0.569	0.098	0.098	0.020	0.020	0.039	-	-	0.020	-	-	0.020	-	-	-	-	-
MJO10-02	0.531	0.184	0.184	-	0.041	-	-	-	0.061	-	-	-	-	-	-	-	-
MJO10-03	0.688	0.104	0.146	0.021	-	0.021	-	-	0.021	-	-	-	-	-	-	-	-
MJO10-04	0.531	0.224	0.204	-	0.020	0.000	-	-	0.020	-	-	-	-	-	-	-	-
MJO11_007	0.500	0.240	0.180	-	-	0.020	-	-	-	-	-	0.060	-	-	-	-	-
MJO11_008	0.580	0.180	0.100	-	0.040	0.020	-	-	0.020	-	-	0.060	-	-	-	-	-
MJO11_009	0.569	0.176	0.098	0.039	0.020	0.039	0.020	-	-	-	-	0.039	-	-	-	-	-
MJO11_010	0.468	0.255	0.064	0.021	0.021	0.085	-	-	0.043	-	-	0.021	-	-	-	-	0.021