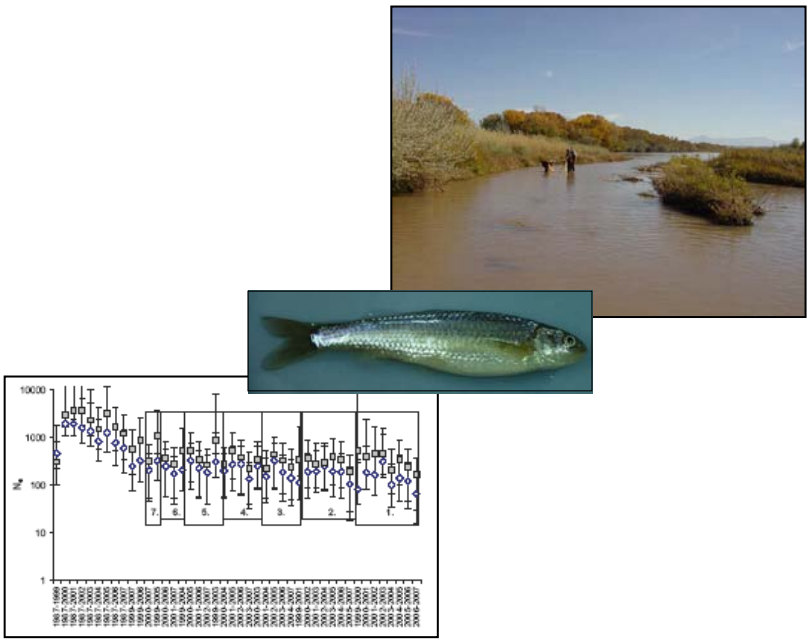
Genetic monitoring of the Rio Grande silvery minnow: Genetic status of wild and captive stocks in 2010.



Annual report FY 2009

Prepared by

Megan J. Osborne and Thomas Turner

Department of Biology and Museum of Southwestern Biology
MSC 03-2020, University of New Mexico
New Mexico, 87131, USA

Submitted to:

U. S. Bureau of Reclamation
Albuquerque Area Office
555 Broadway, NE
Suite 100
Albuquerque, New Mexico.

30 September 2010

Executive Summary

Genetic monitoring is defined as collection of two or more temporally spaced genetic samples from the same population. Temporal sampling allows measurement of changes to various metrics of genetic diversity including allelic richness, heterozygosity, and genetically effective population size (N_e) in contemporary focal populations. This data can be used to track the genetic health of the population and to track impacts of management activities. In addition ecological causes of changes to genetic diversity can be assessed. Genetic monitoring of the Rio Grande silvery minnow using nuclear microsatellites and mitochondrial DNA commenced in 1999 and has continued annually since this time. Here we report on the genetic status of wild and captive stocks of Rio Grande silvery minnow in 2010. In 2010 we sampled 446 wild fish and 497 progeny of captive spawning conducted at Dexter National Fish Hatchery and Technology Center, and the Albuquerque Biological Park. These captive-bred fish represent the stocks released to Big Bend National Park in November 2009.

Major Findings for 2010 are:

(1) Microsatellite gene diversity increased in 2010 from values recorded in previous years and was the highest recorded since genetic monitoring commenced in 1999. In contrast, heterozygosity has declined consistently since 2004, with only a marginal increase in 2010. Allelic richness has remained relatively stable since 2006. Mitochondrial gene diversity in creased in 2010 whilst haplotype richness increased from 2005-2008 values. Several rare haplotypes seen in previous years were not detected in 2010.

(2) Genetic effective size estimates from mitochondrial DNA haploptye frequencies declined for the 2009-2010 time-period to 445 (moments) and 360.6 (MLNE).

(3) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies was higher for the 2009-2010 (Moments N_{eV} = 281, MLNE N_{eV} = 492) temporal comparison than for the previous period 2008-2009. This trend was apparent when either the temporal method or the pseudo-maximum likelihood method was used to estimate effective population size.

(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. Across all captive stocks, a similar number of mitochondrial DNA haplotypes were detected as seen in the wild Middle Rio Grande population.

(5) In 2010 mitochondrial DNA gene diversity and allelic richness lowest in the Isleta reach and higher in the Angostura and San Acacia reaches. For microsatellite data heterozygosity was highest in the Angostura reach whilst AR and gene diversity did not differ greatly between reaches.

Introduction

Sophisticated genetic techniques and analyses are now routinely employed in conservation and management of species listed under the US Endangered Species Act (ESA, as amended in the Federal Register 1973). Most studies are designed to evaluate patterns of genetic divergence in geographic space to identify 'management units' or 'evolutionary significant units' for conservation and recovery planning (Palsboll *et al*. 2007). Genetically distinct populations are likely to be ecologically and evolutionarily independent from other populations. In a practical sense, this means that distinct populations exchange migrants with other populations rarely, if ever, and so management actions applied to one population will have little or no effect on other populations. Genetically distinct populations are also likely to contain uniquely adapted genotypes (and phenotypes) to local habitat conditions and thereby contribute substantially to species recovery and persistence in the wild.

These studies are a cornerstone of conservation genetics and continue to be very important in management; however, they typically provide a static (and historical) rather than dynamic (and contemporary) view of genetic patterns because they depend on samples taken at a single point in time. Once data are in hand, the researcher usually interprets genetic patterns based on evolutionary theory, knowledge of the landscape, and potential for migration between populations (Palsboll *et al*. 2007). This approach does not provide an accurate glimpse into genetic processes of contemporary populations except under limited circumstances. However, most conservation and management plans are carried out at time spans that rarely exceed a few generations of the focal species. Thus, there is considerable interest in developing dynamic genetic research approaches that provide benchmarks and

evaluation of outcome for management and recovery actions in contemporary populations and at contemporary time scales.

Conservation geneticists have recently focused considerable attention on *genetic monitoring* as a potentially powerful tool to reveal connections between demographic and genetic processes in contemporary populations over relevant (i.e., short and contemporaneous) time scales (special issue of the journal *Molecular Ecology*, and a review in *Trends in Ecology and Evolution,* Swartz *et al.* 2007). We follow Swartz *et al*. (2007) and define genetic monitoring as the case where two or more temporally-spaced genetic samples are taken from the same population. Incorporation of temporal sampling offers the advantage of measuring changes in commonly used metrics of genetic diversity such as allelic richness, heterozygosity, and genetically effective population size (N_e) in contemporary focal populations. Rates of genetic and demographic change are intimately linked (Avise 2000), so it is theoretically possible to relate genetic data and metrics to recovery benchmarks like the minimum number of individuals required to stem loss of diversity.

In 1999, we began a genetic monitoring program of the Rio Grande silvery minnow, *Hybognathus amarus* (Girard 1856), five years after the species was listed as federally endangered under the ESA because of precipitous declines in abundance and geographic range size (Federal Register 1994). This genetic monitoring program has continued annually since 1999. During this time the wild population of Rio Grande silvery minnow has undergone dramatic fluctuations (order of magnitude increases and decreases) in abundance (Dudley and Platania 2008). Large declines in abundance are likely accompanied by reductions in genetic effective size that results in accelerated loss of genetic diversity through increases in genetic drift between generations. The rate at which diversity is lost is directly proportional to the genetic effective size of the population. Genetic effective size is defined as the number of individuals that successfully contribute genes to subsequent generation. In most species N_e is smaller than the actual number of individuals in a population however in wild population of Rio Grande silvery minnow we have shown that N_{eV} is orders of magnitude less than the census size (Alò and Turner 2005; Turner and Osborne 2005, 2006).

In this report, we describe genetic analysis of the wild Rio Grande silvery minnow population with temporal samples spanning twelve consecutive years. These data provide unique insight into trends in genetic diversity, causes of loss of diversity, and genetic effects of repatriation of hatchery-reared fishes; all of which are major issues with regard to continued persistence and recovery of this species in the wild. Additionally, we report on the genetic status of the Rio Grande silvery minnow used to reestablish the species in the Big Bend National Park.

Methods

Sampling- Rio Grande Population

Rio Grande silvery minnows were sampled in the Rio Grande annually from 1999 to 2010 (between December and April – just prior to reproduction). 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 33 different captive stocks (seven stocks from captive-reared wild caught eggs and 26 stocks from captive spawning) sampled between 2000 and 2009. In 2009 we screened 478 wild caught Rio Grande silvery minnow and eight groups of fish which were the progeny of captive spawning conducted at Dexter National Fish Hatchery and Technology Center and the Albuquerque Biological Park. Captive-reared fish screened in 2010 were released at four localities (Rio Grande Village, Adams Ranch, Grassy Banks, Santa Elena Canyon) in the Big Bend National Park, Texas in November 2009. These are samples MJO09-005, MJO09-006, MJO09-007, MJO09-008, MJO09-009, MJO09-010, MJO09-011, MJO09-012, MJO09-013, MJO09-014.

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 and Genescan 3.1 (Applied Biosystems).

MtDNA-ND4

Individuals were screened for variation in a 295 base pair fragment of the mitochondrial ND4 gene using Single Stranded Conformational Polymorphism (SSCP) analysis and DNA sequencing. A portion of the mtDNA ND4 gene from each individual was amplified in a 10 *µ*L reaction conatining1 *µ*L template DNA, 1 *µ*L 10× reaction buffer, 2 mM MgCl2, 125 *µ*M dNTPs, 0.5 *µ*M 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*. The 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.

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/. Nei's unbiased genetic diversity (Nei 1987), observed heterozygosity and allele frequencies were obtained using this program. The computer program Microchecker (van Oosterhout *et al*. 2004) was used to examine data for scoring errors due to stuttering, presence of large allele dropout and null alleles. For each microsatellite locus and population, allelic richness (A_R), total number of alleles and inbreeding co-efficients (F_{IS}) were obtained using FSTAT version 2.9.3.1 (Goudet 1995). Allelic richness was calculated using the methods described Petit *et al*. (1998). This method allows the number of alleles to be compared among populations independently of sample size (Leberg 2002) and is based on the smallest number of individuals typed for any locus. The 1999 and 1987 samples were excluded from calculations of allelic richness because of the small number of samples in these collections. FSTAT was also used to test for significant differences in diversity parameters between river reaches. The computer package ARLEQUIN (Schneider *et al*. 2000) was used to assess whether there were significant departures from Hardy-Weinberg equilibrium using the procedure of Guo and Thompson (1992). Global tests for linkage disequilibrium (non-random association of loci) were conducted for all pairs of loci using FSTAT. Bonferroni (Rice 1989) correction was applied to account for multiple simultaneous tests. Estimates of unbiased gene diversity (h) and nucleotide diversity (π) were obtained using ARLEQUIN Version 3.0 for mitochondrial DNA data.

Weir and Cockerham's (1984) *F*-statistics were calculated using ARLEQUIN (Schneider *et al*. 2000) to determine the magnitude of differences between wild fish collected in different years and from the three distinct river reaches. F_{ST} is the standardized variance in allele frequencies between populations and is the most commonly used measure of genetic distance between populations. Φ-statistics were calculated from mt-DNA data (Excoffier *et al*. 1992). Φ-statistics are equivalent to F-statistics however they incorporate allele frequencies and evolutionary distances between haplotypes. Hierarchical analysis of variance (AMOVA) (Excoffier *et al*.1992) partitions the total variance into covariance components due to differences among groups of populations ($F_{CT,}$ Φ_{CT}), between populations within groups ($F_{SC,}$ Φ_{SC}) and among all populations (irrespective of groups) (F_{ST}). Hierarchical analysis of molecular variance was conducted using the wild fish data to partition genetic variance into components attributable to divergence among years ($F_{CT,}$ Φ_{CT}) and between river reaches within years (F_{SC}, Φ_{SC}). A second AMOVA was conducted to test whether a significant proportion of genetic variation could be partitioned into components attributable to differences among wild, captive spawned, and captive reared stocks ($F_{CT,}$ Φ_{CT}), between captive stocks spawned at different times, and

wild caught eggs collected in different years ($F_{SC,}$ Φ_{SC}) and among all populations and captive stocks ($F_{ST,}$ Φ_{ST}). Pairwise F_{ST}s were calculated among the eight stocks released at Big Ben National Park. P-values for all statistics were generated using a bootstrapping method (10,000 permutations).

Estimation of Genetic Effective Size

Variance genetic effective size (N_e) and 95% confidence intervals (CIs) were estimated from temporal changes in microsatellite allele frequencies across year classes using the temporal method (Nei & Tajima 1981; Waples 1989) as implemented in the program NeEstimator (Peel *et al*. 2004) and a pseudo-maximum likelihood procedure implemented in the program MLNE version 2.3 (Wang 2001). *Lco8* was excluded from calculation of N_e because this marker was consistently out of HWE. For mtDNA data (analyzed separately), variance effective size for the female portion of the population (N_{ef}) was estimated with the temporal and pseudo-maximum-likelihood methods. Sampling localities were pooled by year-class prior to analysis. We assumed that genetic sampling did not change the available pool of reproductive individuals and that migration from outside the study area did not affect estimates of N_e. Upstream migration is negligible because dams prohibit fish movement and therefore Rio Grande silvery minnow are rarely taken upstream of the study area.

Temporal-method estimates of N_e and N_{ef} were calculated from *F'* values obtained from all possible pairs cohorts sampled from 1987 to 2009, where *F'* is the standardized variance of allele frequency shifts across cohort pairs corrected for sampling error. MLNE estimates were also based on comparisons of all adjacent cohorts. In all estimates, 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 population monitoring data of R. K. Dudley and S. P. Platania). Consecutive estimates of N_e and N_{ef} were corrected for over-lapping generations (Turner *et al*. 2006; Osborne *et al*. 2010).

Results

Microsatellites- Genetic Diversity

To date, we have characterized microsatellite diversity in 6047 Rio Grande silvery minnow collected from the wild in 1987 and annually between 1999 and 2010 and from silvery minnow spawned and/or reared in captivity and repatriated to the middle Rio Grande, NM and to Big Bend National Park in Texas. Monitoring of captive stocks has been conducted since the beginning of the augmentation program in 2002. Here we report on data collected in 2010 and

Table 1. Sample sizes, collection localities on the Rio Grande, river reaches for wild Rio Grande silvery minnow samples collected in 2010.

River Reach	Locality	Sample Size
Angostura	Bernalillo	34
Angostura	Sandia Line 14	30
Angostura	AMAFCA Channel	11
Angostura	Lomitas Negras	2
Angostura	Calabicillas Arroyo	18
Angostura	Central Ave Bridge	51
Angostura	Atrisco	5
Isleta	Below Isleta DD	12
Isleta	Alejandro Drain	36
Isleta	Los Lunas	50
Isleta	Peralta	48
San Acacia	2 m downstream San Acacia Diversion Dam	50
San Acacia	San Antonio	50
San Acacia	San Marcial	49

Table 2. Summary statistics for microsatellite and mtDNA – ND4 loci for wild (1987, 1999-2010), hatchery reared wild-caught eggs (WcE, An- Angostura, SA- San Acacia, numerals following refer to the years eggs were collected, for example WcE-SA-01 were wild-caught eggs collected from the San Acacia reach in 2001), captively spawned Rio Grande silvery minnow. Sample size (*N*), expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (A_R) and average weighted inbreeding co-efficient (F_{IS}) are given over all loci. For ND4 sample size (N), gene diversity (*h*), allelic (haplotype) richness (A_R) and observed number of haplotypes are given. *WcE-01 sample was also collected from San Acacia but reared at Dexter (WcE-SA-01 was reared at the Biopark).

	Microsatellites					Mt-DNA			
Population	**N**	**H_E**	**H_O**	**F_{IS}**	**A_R**	**N**	***h***	**A_R**	**No. Haps**
1987	43	0.797	0.710	0.111	-	37	0.734	6.000	6
1999	46	0.815	0.647	0.210	-	44	0.427	4.976	5
2000	194	0.815	0.697	0.145	13.298	127	0.389	4.968	6
2001	128	0.808	0.721	0.107	13.729	121	0.610	8.049	10
2002	389	0.794	0.680	0.143	13.676	379	0.630	5.840	8
2003	169	0.818	0.709	0.134	13.902	167	0.524	7.106	9
2004	162	0.820	0.738	0.100	13.792	164	0.612	8.152	9
2005	394	0.817	0.725	0.113	13.947	396	0.610	7.942	10
2006	383	0.826	0.726	0.122	14.040	376	0.621	7.664	10
2007	218	0.829	0.727	0.123	13.821	218	0.579	7.508	10
2008	479	0.824	0.712	0.137	14.074	467	0.572	7.641	11
2009	478	0.832	0.690	0.171	14.046	471	0.592	8.070	12
2010	446	0.839	0.692	0.175	14.201	446	0.649	8.184	9
WILD-CAUGHT EGGS									
WcE-01*	178	0.8199	0.6512	0.206	13.7661	157	0.627	6.999	8
WcE-SA-01	50	0.8305	0.7272	0.126	13.0384	51	0.624	6.000	6
WcE-An-02	50	0.7843	0.7303	0.07	11.0649	49	0.481	2.949	3
WcE-SA-02	81	0.8190	0.6796	0.171	13.9069	81	0.702	7.376	8
WcE-SA-03	51	0.8302	0.6955	0.164	13.8684	51	0.714	7.848	8
MJO-07-005	54	0.8271	0.7387	0.108	13.8007	53	0.602	6.733	7
MJO-07-006	49	0.8143	0.7227	0.114	14.1714	46	0.581	5.962	6
CAPTIVE SPAWNED									
MJO-06-29	50	0.8037	0.7449	0.074	10.3939	50	0.517	5.000	5
Cs-01	64	0.7943	0.6587	0.172	11.9313	58	0.460	4.982	5
Cs-An-02	51	0.6856	0.6754	0.015	7.5074	51	0.000	1.000	1
Cs-SA-02	53	0.8029	0.6733	0.163	12.0341	53	0.751	5.919	6
TFT039	51	0.8060	0.7000	0.133	11.6912	51	0.558	3.995	4
Cs-04	50	0.8237	0.6906	0.163	13.2474	47	0.586	5.911	6
TFT-04-23	50	0.7790	0.6831	0.124	11.0714	48	0.593	4.996	5
TFT-04-24	48	0.8280	0.7170	0.135	11.0870	48	0.609	4.949	5
TFT-04-25	50	0.8100	0.7677	0.053	10.6607	50	0.702	5.934	6
TFT-04-29	54	0.8393	0.7627	0.092	13.0282	54	0.609	4.903	5
TFT-04-30	56	0.8259	0.7265	0.121	13.5240	55	0.656	4.790	5
TFT-04-31	50	0.8046	0.7006	0.13	11.9984	50	0.706	6.865	7
TFT-05-006	50	0.7923	0.6487	0.183	9.7682	50	0.625	5.803	6
TFT-05-007	49	0.7969	0.7045	0.117	11.3052	49	0.550	4.884	5
TFT-05-008	50	0.8044	0.6628	0.178	10.5838	50	0.611	4.934	5
TFT-05-009	50	0.8043	0.7174	0.109	11.8988	50	0.506	3.996	4
TFT-05-011	51	0.8078	0.6925	0.144	11.4467	51	0.573	5.853	6

Table 2 cont.

	Microsatellites					Mt-DNA			
Population	**N**	**H_E**	**H_O**	**F_{IS}**	**A_R**	**N**	***h***	**A_R**	**No. Haps**
MJO-06-25	50	0.8136	0.7208	0.115	13.2821	49	0.635	4.934	5
MJO-06-028	50	0.8051	0.7050	0.125	11.2947	50	0.738	4.996	5
MJO-07-007	50	0.8127	0.7394	0.091	11.9933	50	0.605	4.869	5
MJO-08-006	50	0.8265	0.6694	0.1920	13.4926	45	0.664	6.939	7
MJO-08-007	50	0.8411	0.7213	0.1440	13.1048	50	0.625	6.803	7
MJO-08-008	50	0.8344	0.7113	0.1490	13.3771	49	0.706	5.997	6
MJO-08-009	51	0.8431	0.7151	0.1530	13.7976	51	0.658	5.995	6
MJO-09-001	68	0.8185	0.7059	0.1380	13.6061	62	0.594	8.329	9
MJO-09-002	72	0.7985	0.6700	0.1620	13.3223	68	0.540	6.431	7
MJO-09-003	71	0.8112	0.7187	0.1150	13.1198	64	0.619	5.818	6
MJO-09-004	69	0.8171	0.7134	0.1280	13.2282	64	0.436	5.595	6
MJO-09-005	50	0.8267	0.6905	0.1660	13.8879	49	0.735	5.000	5
MJO-09-006	50	0.8209	0.7061	0.1410	12.9870	50	0.530	4.000	4
MJO-09-007	50	0.8228	0.7034	0.1460	13.3470	51	0.675	5.680	6
MJO-09-008	50	0.8197	0.7119	0.1330	13.7310	50	0.776	6.803	7
MJO-09-009	50	0.8203	0.6975	0.1510	13.3468	50	0.504	7.799	8
MJO-09-010	48	0.8158	0.6975	0.1460	12.7156	43	0.681	8.928	9
MJO-09-011	50	0.7926	0.6566	0.1730	11.2584	49	0.767	5.000	5
MJO-09-012	49	0.8027	0.6747	0.1610	11.5320	43	0.666	7.000	7
MJO-09-013	50	0.8110	0.6698	0.1760	12.2283	50	0.563	7.792	8
MJO-09-014	50	0.8037	0.6814	0.1540	12.7793	47	0.742	7.955	8

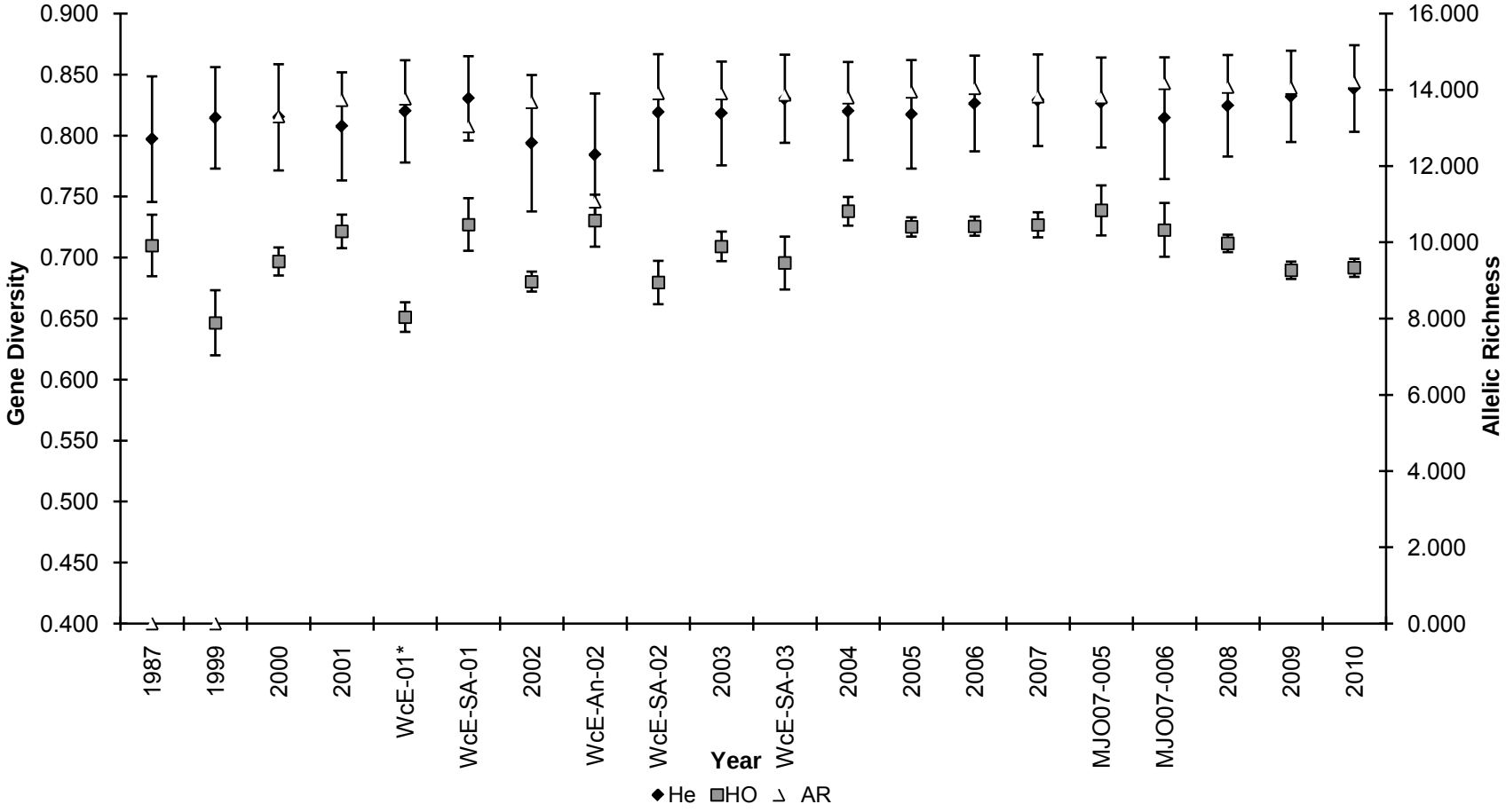


Figure 1. Microsatellite diversity statistics for wild populations(by year) and for captive stocks reared from wild-caught eggs. Gene diversity (H_e), heterozygosity (H_O) and allelic richness (A_R) are given. Standard deviations are given for H_e and H_O.

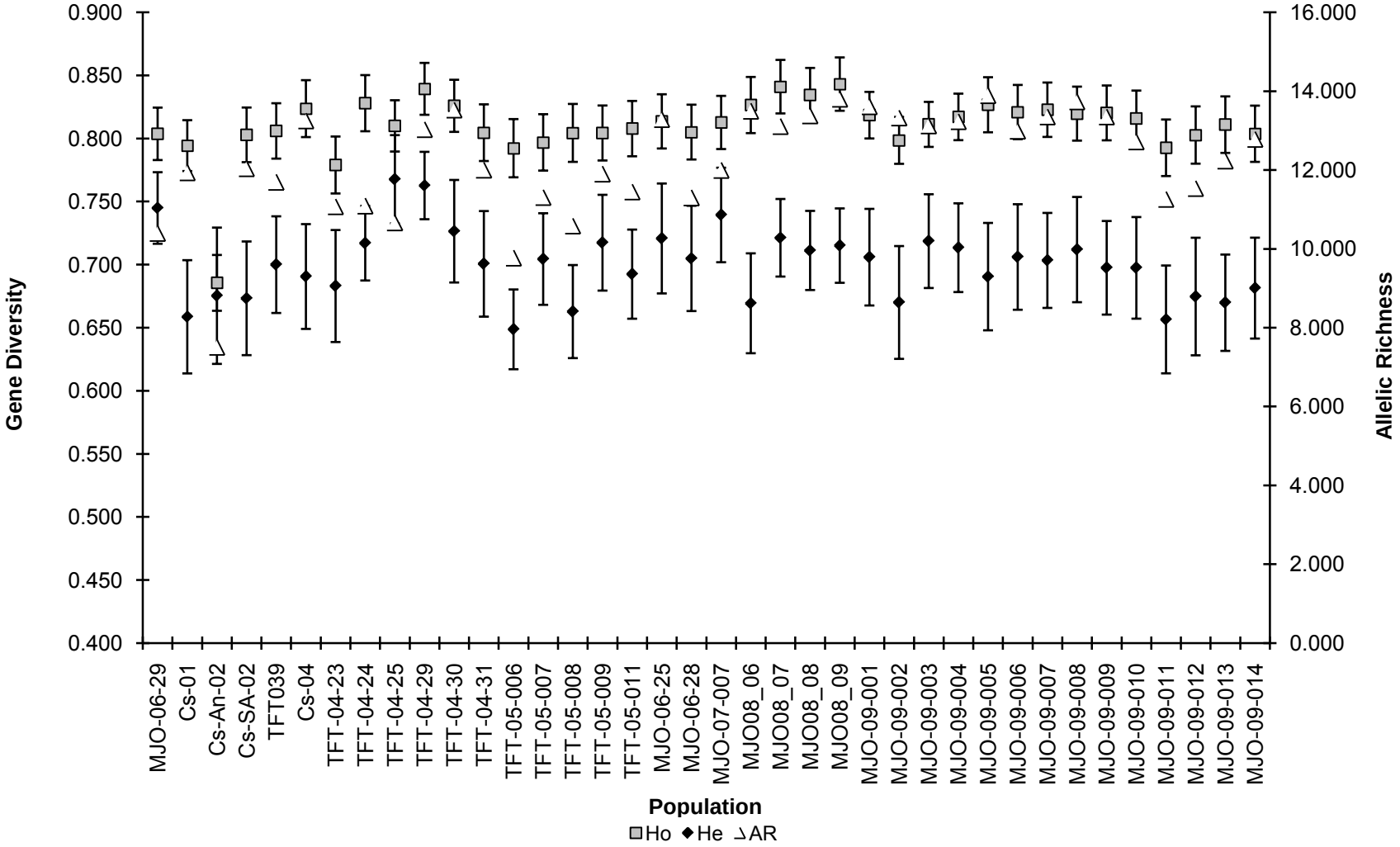


Figure 2. Microsatellite diversity statistics for captive stocks. Gene diversity (H_e), heterozygosity (H_O) and allelic richness (A_R) are given. Standard deviations are given for H_e and H_O.

Table 3. Mt-DNA haplotype frequencies across all wild and captive stocks.

	Mt-DNA-ND4 Haplotypes															
	A	**C**	**D**	**E**	**F**	**K**	**I**	**J**	**M**	**N**	**P**	**O**	**Q**	**S**	**T**	**W**
1987	0.459	0.189	0.162	0.054	0.081	-	-	-	0.054	-	-	-	-	-	-	-
1999	0.750	-	0.114	0.068	0.045	0.023	-	-	-	-	-	-	-	-	-	-
2000	0.772	0.008	0.047	0.071	0.094	0.008	-	-	-	-	-	-	-	-	-	-
2001	0.607	0.090	0.057	0.033	0.107	0.066	0.008	0.016	0.008	-	-	0.008	-	-	-	-
2002	0.538	0.203	0.148	0.011	0.061	0.034	-	0.003	-	-	-	0.003	-	-	-	-
2003	0.671	0.054	0.150	0.030	0.054	0.012	-	0.006	0.006	-	-	0.018	-	-	-	-
2004	0.604	0.085	0.104	0.018	0.073	0.049	0.012	-	0.018	-	-	0.030	-	-	-	-
2005	0.598	0.126	0.088	0.028	0.086	0.018	0.015	0.003	0.028	-	-	0.010	-	-	-	
2006	0.588	0.135	0.092	0.047	0.047	0.047	0.003	-	0.029	-	-	0.008	-	-	0.003	-
2007	0.628	0.110	0.083	0.023	0.087	0.037	0.005	-	0.005	-	-	0.018	0.005	-	-	-
2008	0.629	0.121	0.080	0.026	0.067	0.046	0.007	-	0.009	-	0.002	0.007	-	0.007	-	-
2009	0.616	0.140	0.0764	0.0276	0.0637	0.034	0.006	0.002	0.019	-	0.002	0.011	-	0.002	-	-
2010	0.564	0.125	0.097	0.030	0.069	0.053	0.014	-	0.016	-	-	0.032	-	-	-	-
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	0.020	0.327	-	-	-	-	-		-	-	-	-	-	-	-
WcE-SA02	0.488	0.225	0.050	0.013	0.138	0.050	-	-	0.038	-	-	-	-	-	-	-
WcE-SA-03	0.490	0.078	0.196	0.059	0.098	0.039	-	-	0.020	-	-	0.020	-	-	-	-
MJO07-005	0.604	0.094	0.019	0.019	0.170	0.075	-	-	-	-	-	-	-	-	-	-
MJO07-006	0.630	0.087	0.130	0.022	0.087	0.043	-	-	-	-	-	-	-	-	-	-
MJO06-29	0.680	0.140	0.080	-	0.060	-	-	-	0.040	-	-	-	-	-	-	-
Cs-01	0.724	0.052	-	0.034	0.069	0.121	-	-	-	-	-	-	-	-	-	-
Cs-An-02	-	-	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-
Cs-SA-02	0.434	0.075	0.170	0.132	0.170	-	-	-	-	-	-	-	-	-	-	-
Cs-04	0.596	0.255	0.021	-	0.043	0.064	-	-	-	-	-	0.021	-	-	-	-
TFT039	0.596	0.269	0.038	-	-	0.096	-	-	-	-	-	-	-	-	-	-

Table 3 cont.

	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T	W
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	0.400	0.333	-	-	-	0.244	-	-	-	-	0.022	-	-	-	-	-
TFT04-31	0.420	0.340	0.020	-	0.060	0.040	-	-	0.100	-	-	0.020	-	-	-	-
TFT05-06	0.500	0.360	0.020	-	0.020	0.080	-	-	0.020	-	-	-	-	-	-	-
TFT05-07	0.625	0.292	0.021	0.063	-	-	-	-	-	-	-	-	-	-	-	-
TFT05-08	0.592	0.082	-	0.102	-	0.224	-	-	-	-	-	-	-	-	-	-
TFT05-09	0.680	0.160	-	-	-	0.120	-	-	0.040	-	-	-	-	-	-	-
TFT05-11	0.623	0.057	0.113	0.019	0.170		-	-	0.019	-	-	-	-	-	-	-
MJO06-25	0.551	0.245	0.061	-	0.061	0.082	-	-	-	-	-	-	-	-	-	-
MJO06-28	0.400	0.140	0.220	-	0.220	0.020	-	-	-	-	-	-	-	-	-	-
MJO07-007	0.560	0.020	0.120	0.020	0.280	-	-	-	-	-	-	-	-	-	-	-
MJO08_06	0.533	0.222	0.044	0.044	0.111	0.022	-	-	-	-	-	0.022	-	-	-	-
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	0.613	0.177	0.048	0.016	0.065	0.032	-	-	0.016	-	-	0.016	-	-	-	0.016
MJO09_02	0.647	0.206	0.074	0.015	0.015	0.015	0.029	-	0.000	-	-	-	-	-	-	-
MJO09_03	0.578	0.203	0.063	-	0.094	0.016	-	-	0.047	-	-	-	-	-	-	-
MJO09_04	0.734	0.172	0.031	-	0.031	0.016	-	-	0.016	-	-	-	-	-	-	-
MJO09_05	0.449	0.163	0.163	-	0.122	0.102	-	-	-	-	-	-	-	-	-	-
MJO09_06	0.660	0.080	0.080	-	0.180	-	-	-	-	-	-	-	-	-	-	-
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	-	-	-	-	-	-	-	-	-
MJO09_14	0.468	0.106	0.064	0.064	0.170	0.043	-	-	-	-	0.021	0.064	-	-	-	-

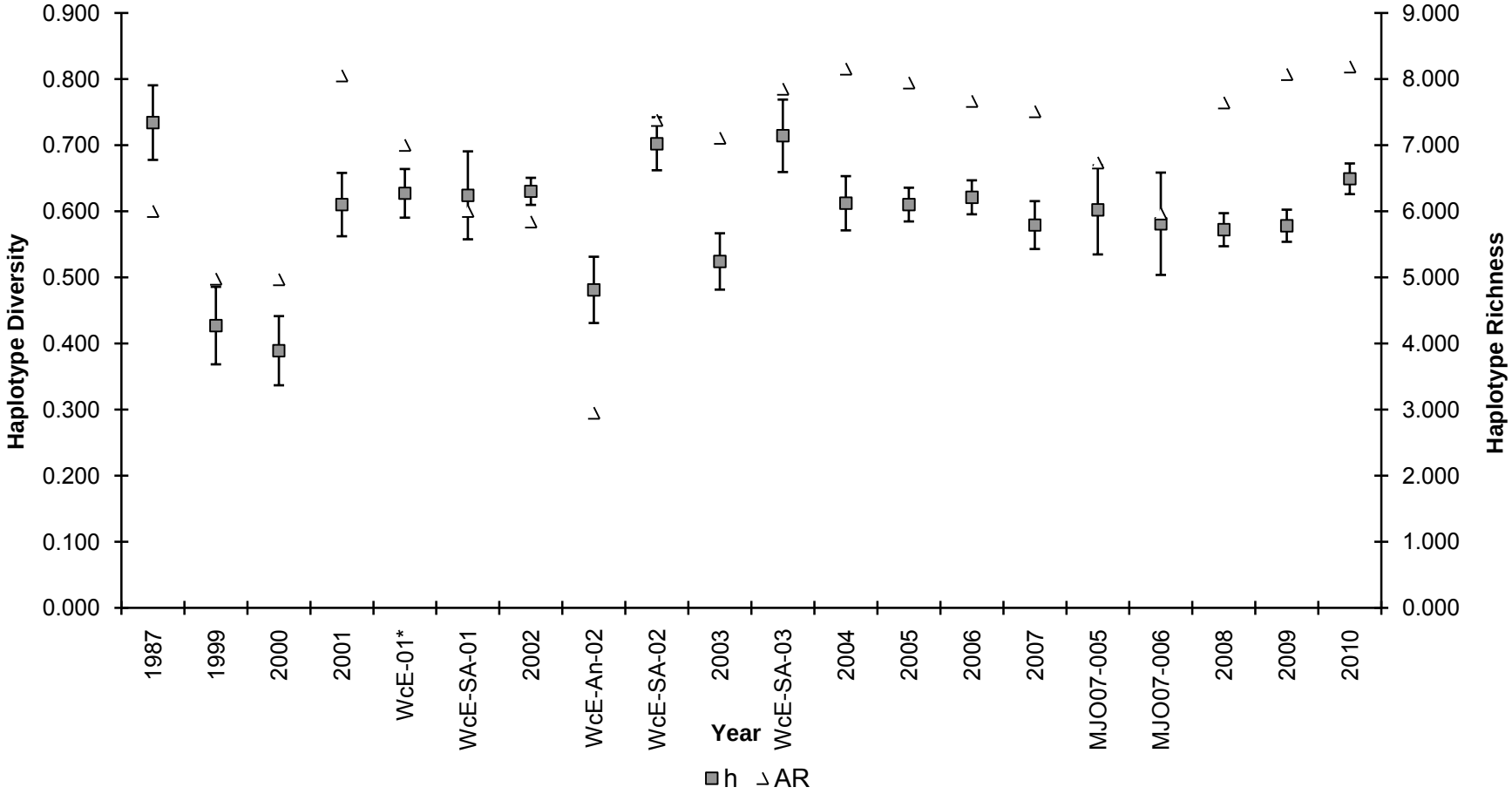


Figure 3. Mt-DNA diversity statistics for wild populations(by year) and for captive stocks reared from wild-caught eggs. Gene diversity (*h*), and haplotype richness (A_R) are given. Standard deviations are given for *h*.

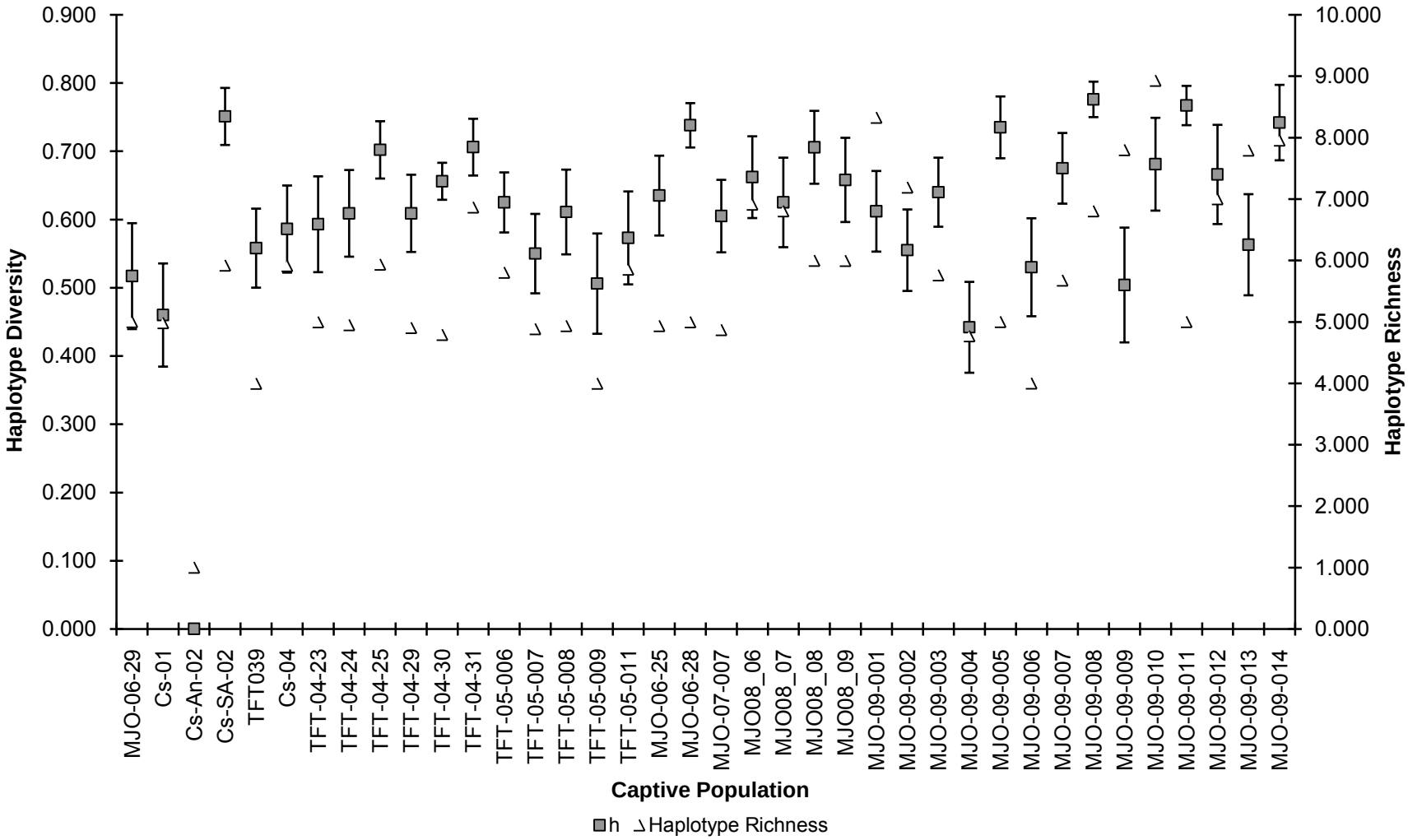


Figure 4. Mt-DNA diversity statistics for captive stocks. Gene diversity (*h*), and haplotype richness (A_R) are given. Standard deviations are given for *h*.

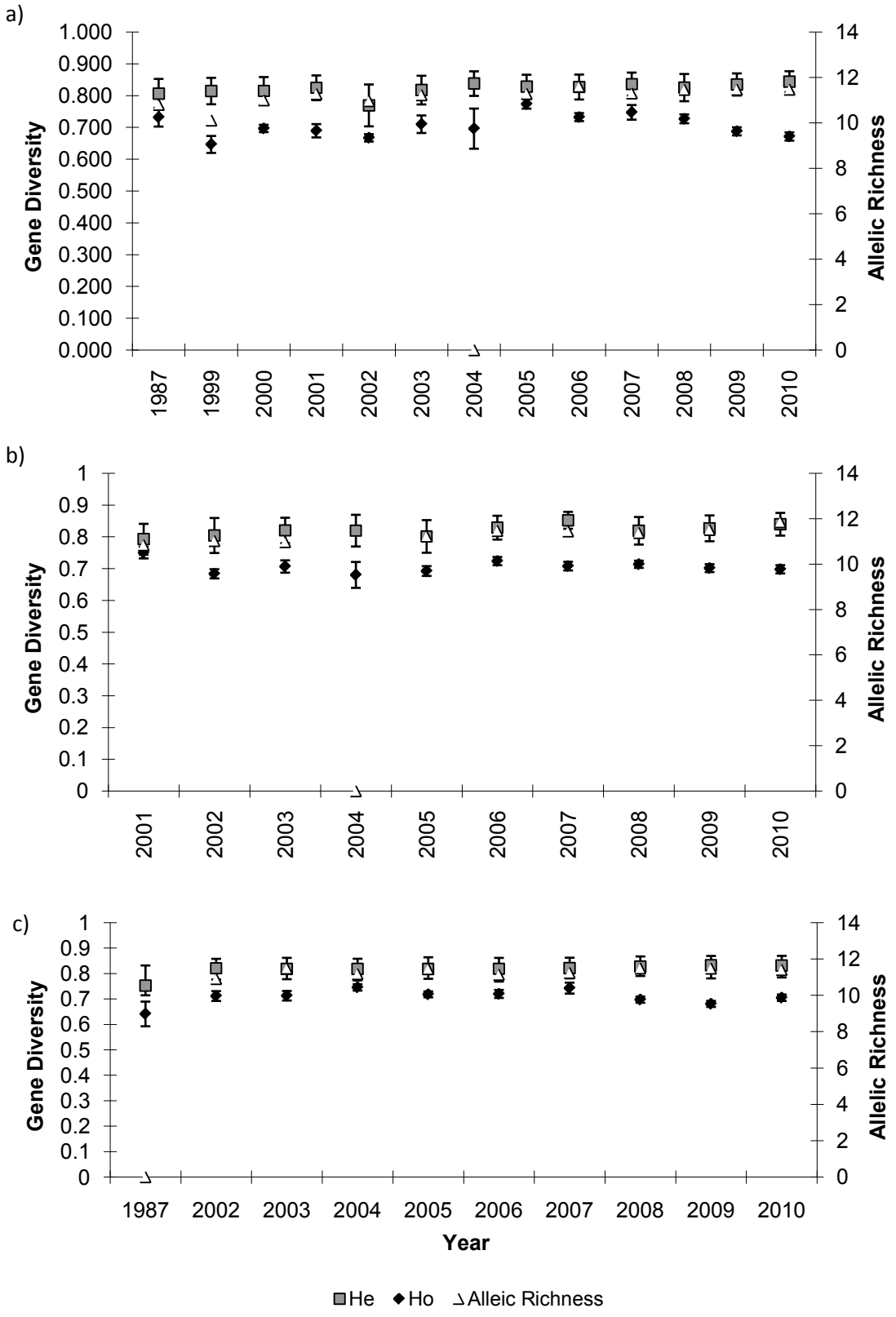


Figure 5. Microsatellite diversity statistics by year and river reach a) Angostura, b) Isleta and c) San Acacia. Gene diversity (H_e), observed heterozygosity (H_o) and allelic richness (A_R) are given.

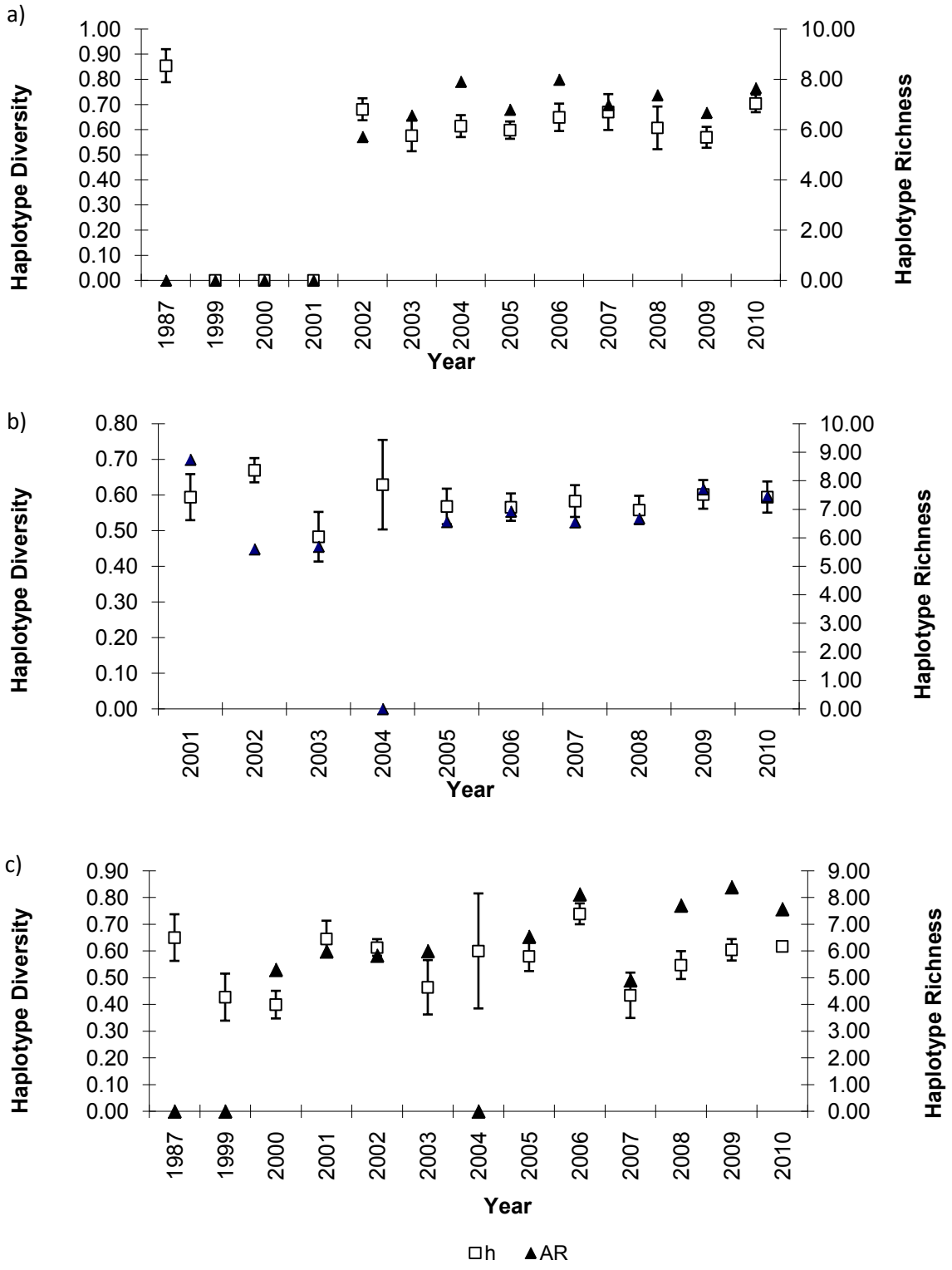


Figure 6. MtDNA diversity statistics by year and river reach a) Angostura, b) Isleta and c) San Acacia. Haplotype diversity (h), and haplotype richness (A_R) are given. Zeros indicate either no samples or sample size insufficient to estimate A_R.

compare these to previous data. In 2010, Rio Grande silvery minnow were sampled from 14 localities representing the Angostura, Isleta and San Acacia reaches of the middle Rio Grande (Table 1), New Mexico.

After Bonferroni correction for multiple comparisons there were 152 departures from Hardy-Weinberg equilibrium from a total of 522 comparisons. Forty-eight of these involved wild samples, 22 involved fish reared from wild-caught eggs and 82 involved captive spawned stocks. Across all samples there was 14 cases of linkage disequilibrium among loci.

Wild individuals collected in 2010 exhibited similar levels of genetic diversity at nearly every measure compared to wild fishes in 2009 (Table 2, Figure 1). In 2010, observed heterozygosity (H_O) increased marginally from values seen in 2009 to 0.6916. Allelic richness increased slightly from that recorded in 2009 and was higher than values recorded from 2000-2007. In nearly all cases, allelic richness and gene diversity in captive spawned stocks (Figure 3) were lower than in stocks reared from wild-caught eggs (Figure 2). On average, heterozygosity was higher and average inbreeding co-efficients were lower in the captive-spawned stocks than those reared from wild-caught eggs.

Average diversity measures were compared between wild, wild-caught eggs and captive spawned stocks using t-tests. Average allelic richness (P = 0.036) differed significantly between these groups whilst gene diversity (P = 0.425), heterozygosity (P = 0.537) and average inbreeding co-efficients (P= 0.489) however were not significantly different among groups. Allelic richness declined slightly in 2010 in the Angostura reach compared to 2009 values whilst A_R increased in the Isleta reaches. Heterozygosity increased in the Angostura and Isleta reaches in 2010 compared to values recorded in 2009 (Figure 5a-c).

Mt-DNA- Genetic Diversity

Nine ND4 haplotypes were identified in wild Rio Grande silvery minnow collected in 2010. As observed in previous years, haplotype A was the most frequently encountered (Table 3). Seven haplotypes were present in fewer than 10% of individuals. Gene diversity (*h*) in the wild 2010 sample increased over previous years although fewer haplotypes were identified than from 2005-2009 (Table 2, Figure 3). In 2010 diversity (*h*) was higher in fish collected from the Angostura reach than those collected from the Isleta and San Acacia reaches. Eight haplotypes were detected in the Angostura and Isleta reaches whilst nine haplotypes were seen in fish collected from San Acacia reaches (Figure 6). Allelic richness was very similar among reaches. In the Angostura and San Acacia reaches gene diversity increased in 2010 compared to values recorded in 2009 whilst it decreased in the Isleta reaches from 2009 (Figure 6a-c).

Captive reared fish released to Big Bend National Park, Texas

Measures of genetic diversity (H_E, H_O) for captive spawned stocks that were released at Big Bend National Park were comparable to diversity statistics calculated for the Middle Rio Grande wild population. Allelic richness was slightly lower in the captive=reared fish released to Big Bend when compared to the wild, Middle Rio Grande population**.** The number of MtDNA haplotypes identified in these captive stocks ranged from four to nine (Figure 4). Four sampled (MJO09-009, MJO09-010, MJO09-013, MJO09-014) had haplotype richness values that were similar to the wild samples whilst the rest were generally lower. Gene diversity ranged from 0.504 to 0.776. Pairwise F_{STs} calculated among the ten lots of captive-spawned fish revealed no significant variation among the four lots of fish released from Dexter National Fish Hatchery (MJO09-005, MJO09-006, MJO09-007, MJO09-008) but for fish released from the Albuquerque Biological Park, there were ten significant F_{ST} values among lots after Bonferroni correction was applied. Mean F_{ST} among lots released from Dexter was 0.003 whilst for lots released from the Albuquerque Biological Park, mean F_{ST} was 0.0129. Values of Φ_{ST} calculated among all pairs of captive-bred fish released to Big Bend in 2010 were small and two significant comparisons were identified.

Microsatellites- Population Structure

Pairwise values of F_{ST} were calculated between all temporal samples collected from the middle Rio Grande since project inception. Values of pairwise F_{ST} were relatively small but 10 of 78 total comparisons were significant after Bonferroni correction (Table 4a). Hierarchical analysis of molecular variance was conducted by grouping samples by river reach across all years. Values were not significantly different from zero, indicating that river reach did not explain a significant portion of genetic variance (F_{CT}=0.0002, P= 0.4408). Pairwise F_{STs} also were calculated for 2010 among sampling localities. Values of F_{ST} were small and none were significantly different from zero after Bonferroni correction for multiple tests (Table 4c).

Mt-DNA- Population Structure

Pairwise Φ-statistics were calculated between all wild samples collected in 1987 and from 1999-2009. After Bonferroni correction was applied, three significant comparisons were identified from a total of 66 comparisons (Table 4b). The significant comparisons involved the 2002 sample. We also conducted two hierarchical analyses of molecular variance in which samples were grouped by year and by river reach. Results indicated that genetic differences among river reaches (Angostura, Isleta and San Acacia) (Φ_{CT} = -0.0009, P = 0.837) were not significantly different from zero. When samples were grouped by years a small but significant (Φ_{CT} =

Table 4. **a**) Pairwise F_{STs} calculated from microsatellite data among wild, temporal samples (below diagonal) and P-values (above diagonal). **b**) Pairwise Φ_{STs} calculated from Mt-DNA-ND4 data among wild, temporal samples (below diagonal). **c)** Pairwise F_{STs} calculated among sampling localities for microsatellites and **d)** mitochondrial DNA. Shading indicates significant values after Bonferroni correction. DSADD- downstream San Acacia Diversion Dam.

a)

	1987	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
1987	*	0.9998	0.99881	0.99921	0.9891	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998
1999	-0.0316	*	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998
2000	-0.0067	-0.0275		0.1819	0.0000	0.9155	0.8512	0.9962	0.0014	0.0163	0.0722	0.9996	0.9334
2001	-0.0088	-0.0268	0.0009	*	0.0000	0.9998	0.9998	0.9998	0.2976	0.0411	0.7570	0.9998	0.9998
2002	-0.0032	-0.0271	0.0038	0.0034	*	0.0002	0.0002	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000
2003	-0.0100	-0.0358	-0.0007	-0.0033	0.0025	*	0.9480	0.9998	0.9998	0.9996	0.9998	0.9998	0.9998
2004	-0.0133	-0.0398	-0.0005	-0.0036	0.0024	-0.0008	*	0.9998	0.9972	0.2993	0.9884	0.0138	0.9998
2005	-0.0113	-0.0256	-0.0012	-0.0023	0.0013	-0.0058	-0.0044		0.9998	0.9879	0.9998	0.9998	0.9998
2006	-0.0108	-0.0276	0.0017	0.0004	0.0044	-0.0034	-0.0011	-0.0011	*	0.00139	0.00456	0.00159	0.00595
2007	-0.0117	-0.0350	0.0015	0.0016	0.0067	-0.0019	0.0005	-0.0008	0.0016	*	0.00119	0.22991	0.9998
2008	-0.0091	-0.0324	0.0008	-0.0002	0.0061	-0.0033	-0.0008	-0.0022	0.0009	0.0015	*	0.9998	0.9998
2009	-0.0178	-0.0379	-0.0009	-0.0014	0.0033	-0.0018	0.0014	-0.0031	0.0011	0.0005	-0.0024	*	0.9998
2010	-0.0185	-0.0274	-0.0004	-0.0024	0.0035	-0.0031	-0.0025	-0.0033	0.0009	-0.0014	-0.0044	-0.0005	*

b)

	1987	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
1987	*	0.145	0.008	0.009	0.273	0.187	0.056	0.035	0.106	0.020	0.008	0.015	0.095
1999	0.020	*	0.384	0.233	0.077	0.688	0.504	0.286	0.358	0.312	0.257	0.224	0.302
2000	0.078	-0.002	*	0.252	0.000	0.029	0.074	0.028	0.009	0.085	0.039	0.014	0.007
2001	0.059	0.004	0.002	*	0.002	0.036	0.355	0.213	0.054	0.588	0.464	0.220	0.068
2002	0.003	0.018	0.049	0.029	*	0.057	0.027	0.004	0.038	0.008	0.000	0.002	0.051
2003	0.010	-0.009	0.019	0.016	0.008	*	0.299	0.088	0.223	0.103	0.031	0.046	0.205
2004	0.027	-0.004	0.011	0.000	0.011	0.000	*	0.731	0.470	0.865	0.662	0.637	0.715
2005	0.031	0.002	0.012	0.002	0.012	0.005	-0.002	*	0.182	0.788	0.370	0.705	0.219
2006	0.016	-0.001	0.021	0.009	0.005	0.002	-0.001	0.001	*	0.161	0.077	0.174	0.728
2007	0.041	0.001	0.008	-0.002	0.015	0.006	-0.004	-0.002	0.002	*	0.941	0.798	0.257
2008	0.049	0.003	0.010	-0.001	0.018	0.009	-0.002	0.000	0.003	-0.003	*	0.818	0.083
2009	0.039	0.004	0.014	0.002	0.013	0.008	-0.002	-0.001	0.001	-0.002	-0.001	*	0.171
2010	0.017	0.001	0.021	0.008	0.005	0.002	-0.002	0.001	-0.001	0.001	0.003	0.001	*

c)

	Bernalillo	Calabicillas	Sandia L14	AMAFCA	Central Ave	Alejandro Drain	Isleta DD	Los Lunas	Peralta	DSADD	San Antonio	San Marcial
Bernalillo	*	0.0052	0.2119	0.2930	0.2509	0.6983	0.2099	0.9998	0.4856	0.3148	0.9903	0.1754
Calabicillas	0.0168	*	0.6905	0.0054	0.0429	0.0508	0.2821	0.9931	0.1972	0.0210	0.1710	0.3936
Sandia L14	0.0046	0.0017	*	0.2861	0.1819	0.7403	0.8631	0.9998	0.0926	0.5354	0.9994	0.4914
AMAFCA	0.0058	0.0307	0.0090	*	0.0357	0.1904	0.2638	0.9988	0.0405	0.2855	0.3866	0.0294
Central Ave	0.0027	0.0099	0.0042	0.0146	*	0.5765	0.4007	0.9998	0.0458	0.0419	0.9998	0.0460
Alejandro Drain	0.0000	0.0120	0.0006	0.0106	0.0011	*	0.4696	0.9998	0.2006	0.4319	0.9919	0.1760
Isleta DD	0.0077	0.0117	-0.0004	0.0136	0.0044	0.0053	*	0.9998	0.1387	0.4852	0.8012	0.3882
Los Lunas	-0.0272	-0.0081	-0.0367	-0.0197	-0.0350	-0.0206	-0.0336	*	0.9998	0.9998	0.9986	0.9998
Peralta	0.0010	0.0054	0.0057	0.0133	0.0047	0.0036	0.0089	-0.0394	*	0.6570	0.9998	0.4874
DSADD	0.0024	0.0121	0.0019	0.0065	0.0050	0.0022	0.0039	-0.0315	0.0004	*	0.9990	0.0482
San Antonio	-0.0057	0.0072	-0.0082	0.0055	-0.0074	-0.0046	-0.0007	-0.0063	-0.0086	-0.0057	*	0.9994
San Marcial	0.0047	0.0053	0.0029	0.0192	0.0057	0.0051	0.0076	-0.0247	0.0018	0.0058	-0.0056	*

d)

	Bernalillo	Calabicillas	Sandia L14	AMAFCA	Central Ave	Alejandro Drain	Isleta DD	Los Lunas	Peralta	DSADD	San Antonio	San Marcial
Bernalillo	*	0.2995	0.2216	0.5779	0.4926	0.3886	0.3981	0.4957	0.6987	0.0436	0.3658	0.3537
Calabicillas	0.0063	*	0.8836	0.1656	0.6080	0.1920	0.3817	0.3343	0.3501	0.6538	0.4335	0.5900
Sandia L14	0.0118	-0.0337	*	0.1914	0.4569	0.1028	0.5080	0.1649	0.2420	0.3232	0.2498	0.3059
AMAFCA	-0.0187	0.0412	0.0265	*	0.3142	0.3031	0.5767	0.5725	0.7274	0.0476	0.4864	0.4225
Central Ave	-0.0057	-0.0147	-0.0040	0.0051	*	0.5174	0.3392	0.3450	0.3975	0.2833	0.5495	0.6354
Alejandro Drain	-0.0027	0.0251	0.0318	0.0028	-0.0068	*	0.2819	0.3015	0.3702	0.1159	0.4398	0.3243
Isleta DD	-0.0017	0.0077	-0.0114	-0.0282	0.0027	0.0072	*	0.2982	0.5326	0.1601	0.4114	0.3936
Los Lunas	-0.0082	0.0030	0.0161	-0.0217	0.0001	0.0012	0.0072	*	0.9528	0.0657	0.7245	0.6679
Peralta	-0.0132	0.0003	0.0090	-0.0284	-0.0020	-0.0017	-0.0177	-0.0188	*	0.0926	0.8863	0.7989
DSADD	0.0420	-0.0171	0.0036	0.0742	0.0041	0.0215	0.0287	0.0330	0.0260	*	0.2773	0.3051
San Antonio	-0.0014	-0.0070	0.0074	-0.0113	-0.0064	-0.0048	-0.0079	-0.0122	-0.0163	0.0046	*	0.9950
San Marcial	-0.0007	-0.0171	0.0033	-0.0064	-0.0091	0.0005	-0.0045	-0.0122	-0.0150	0.0029	-0.0201	*

0.0065, P=0.0009) portion of variation could be explained by year. We also calculated pairwise Φ_{ST} values among 2010 samples collected at different localities. There were no significant differences among localities after Bonferroni correction was applied (Table 4d).

Effective Population Size

Variance effective size was estimated between all wild samples collected from 1999 to 2009 (Figure 6a, Table 5). With the exception of the 1987-1999 comparison, all pairwise comparisons with a sample collected in 1987 all of estimates of N_{eV} were above 1000. This was true for both moments and pseudo-maximum likelihood estimates. For the 2009-2010 comparison (corrected for overlapping generations) N_{eV} was 280.67 (95 % CIs 190.75 – 460.28) and the MLNE estimate was 492.125 (95 % CIs 358.01 – 744.855).

Variance female effective size (calculated from Mt-DNA haplotype frequencies) declined for the most recent temporal comparison (2009-2010) to N_{ef} of 445 and 360.6 (MLNE) (Figure 6b, Table 6).

Discussion

Genetic status of the species in 2010

To interpret genetic data it is important to consider the demographic trends in the population. Over the past few year catch rates for Rio Grande silvery minnow have fluctuated dramatically with substantial declines from 2005 to 2006 and subsequent increases in density in more recent years (2007-2009) associated with elevated spring runoff and more limited episodes of river drying (Dudley et al. 2009). Greater stability in the wild population from 2007-2009 is reflected in genetic diversity estimates. In 2010 average gene diversity increased above that recorded in 2008 and microsatellite allelic richness remained stable from 2006-2010 in the wild Rio Grande silvery minnow population. The level of gene diversity and allelic richness at the mitochondrial ND4 gene was the highest recorded since 1987. However fewer mitochondrial haplotypes were identified in 2010 than from 2005-2009, with five rare haplotypes not detected in 2010. Interestingly, these haplotypes were rarely detected in the captive stocks. Here, the word ‘haplotype’ refers to the matrilineal inheritance of the mtDNA genome, which is distinct from biparental inheritance exhibited by microsatellites (i.e., both a male and female parent contributes to the ‘genotype’).

Estimates (from microsatellite data) of variance effective population size for 2009-2010 calculated using the temporal method, increased over values recorded for the previous period (2008-2009). Despite the increase in genetic effective size it is still a fraction of the estimated

Figure 7. Effective size estimates calculated from (a) microsatellites (Ne) and (b) mitochondrial DNA data. 95% CIs are shown.

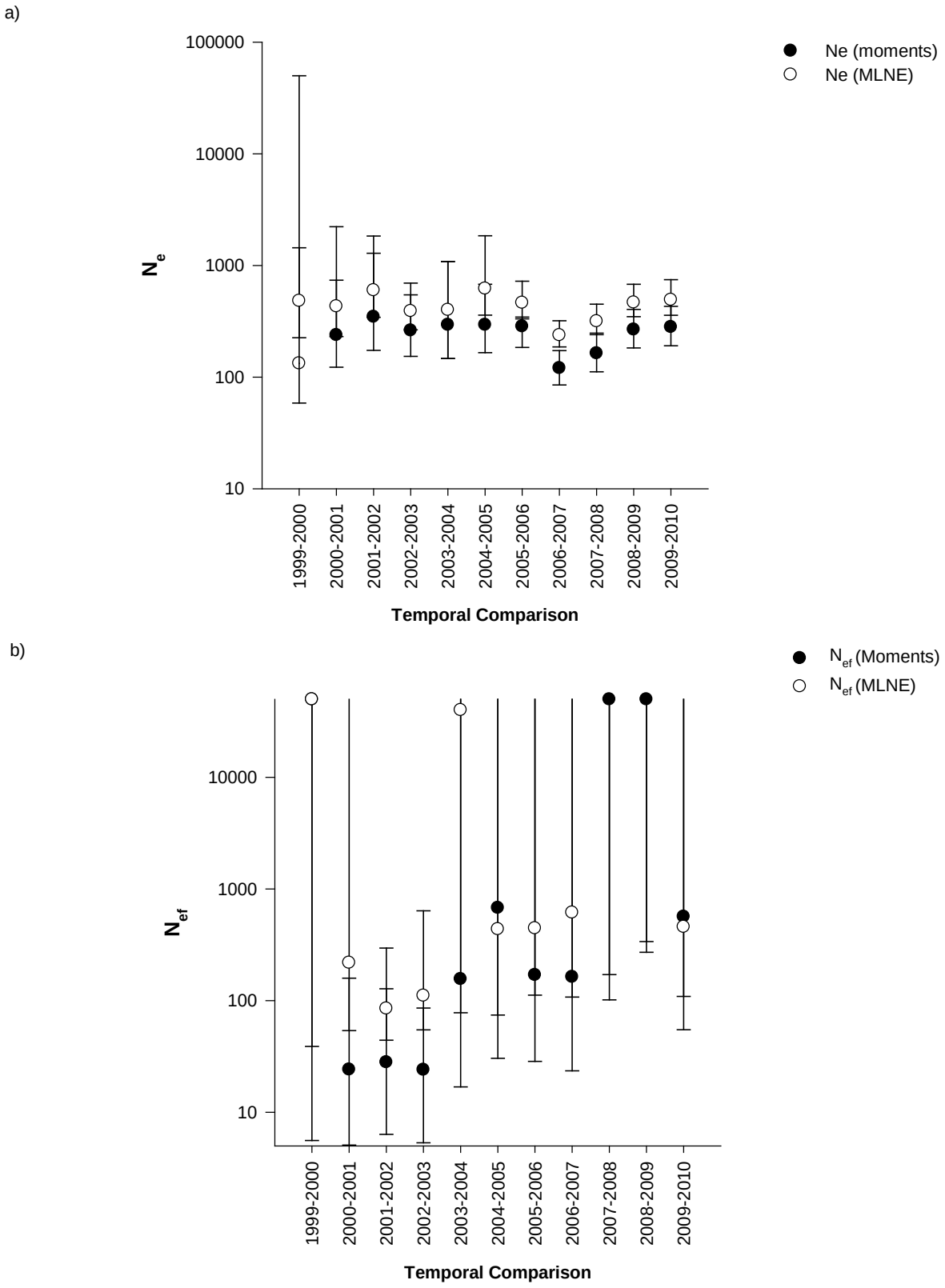


Table 5. Estimates of effective size (N_e) and 95% confidence limits. Consecutive estimates (e.g. 2009-2010) are corrected for overlapping generations.

Generations	Temporal Comparison	$N_{e(moments)}$	-95%	+95%	N_e (MLNE)	-95%	+95%
12	1987-1999	541.2	253.6	2409.1	316.5	205.0	558.7
13	1987-2000	2208.8	791.5	infinity	2455.4	1167.5	infinity
14	1987-2001	6366.9	1171.1	infinity	7250.9	1938.4	infinity
15	1987-2002	7949.5	1478.8	infinity	4904.9	2081.4	infinity
16	1987-2003	5330.3	1247.6	infinity	5218.8	1829.7	infinity
17	1987-2004	3825.5	1159.2	infinity	2858.3	1394.1	16584.9
18	1987-2005	2227.0	1393.9	infinity	4386.4	2097.3	29103.1
19	1987-2006	2227.0	1036.5	13831.1	2919.4	1672.2	7160.2
20	1987-2007	2330.1	1034.8	24302.4	3010.6	1593.8	infinity
21	1987-2008	5605.4	2612.6	44439.5	5605.4	2673.5	infinity
22	1987-2009	3190.4	1363.6	149202.2	4664.4	2409.7	17106.0
23	1987-2010	2520.4	1217.0	11650.6	4011.4	2242.1	12365.2
11	1999-2010	725.0	408.2	1679.4	1181.3	776.6	2040.5
10	1999-2009	746.6	403.3	1973.4	1362.4	846.3	2707.8
10	2000-2010	612.6	451.3	830.7	801.1	630.8	1032.5
9	2000-2009	750.8	535.0	1064.8	1013.8	767.8	1383.8
9	1999-2008	601.2	334.4	1434.4	966.3	637.0	1669.8
9	2001-2010	695.6	483.0	1028.8	912.5	677.5	1278.5
8	1999-2007	929.1	561.8	2024.6	929.1	561.8	2024.6
8	2000-2008	709.9	500.8	1021.4	928.9	697.0	1278.4
8	2001-2009	617.8	428.5	913.6	870.3	642.5	1233.5
8	2002-2010	506.3	385.4	658.6	581.3	475.1	716.3
7	1999-2006	670.4	327.8	2814.4	1222.5	701.3	3187.6
7	2000-2007	535.6	368.8	797.5	669.0	488.3	956.5
7	2001-2008	731.2	481.4	1178.7	905.6	646.4	1345.9
7	2002-2009	576.8	431.4	766.6	671.5	536.7	849.0
7	2003-2010	617.2	432.2	900.2	797.9	596.7	1112.5
6	1999-2005	532.4	266.5	1939.0	1090.1	610.3	2936.5
6	2000-2006	632.8	433.2	954.5	906.5	653.3	1338.8
6	2001-2007	414.3	279.7	637.0	530.3	382.2	779.3
6	2002-2008	488.9	365.0	651.2	545.3	437.7	687.1
6	2003-2009	530.7	371.5	773.3	731.6	545.9	1019.2
6	2004-2010	618.7	424.9	931.0	903.5	646.3	1354.2
5	1999-2004	232.5	135.1	481.1	394.5	260.9	688.8
5	2000-2005	1338.8	742.1	3243.2	1969.5	1100.9	5415.0
5	2001-2006	733.0	446.3	1383.3	957.1	631.4	1669.5
5	2002-2007	334.4	245.1	456.2	379.9	300.8	491.1
5	2003-2008	518.4	353.1	787.9	640.6	472.1	912.1
5	2004-2009	466.7	324.6	686.9	743.8	536.6	1100.6
5	2005-2010	445.1	330.4	597.9	525.3	419.0	668.3
4	1999-2003	314.2	154.8	1206.3	684.4	357.1	2744.2
4	2000-2004	424.0	268.6	729.5	511.5	344.8	858.7
4	2001-2005	855.4	464.1	2227.0	1087.5	646.5	2408.4
4	2002-2006	462.2	332.1	64934.0	546.9	421.9	726.0
4	2003-2007	320.4	218.5	486.1	389.0	285.7	558.5
4	2004-2008	402.8	275.7	607.1	612.4	438.9	912.4
4	2005-2009	398.9	294.1	540.6	489.8	388.8	628.0
4	2006-2010	383.1	283.4	517.4	461.0	365.7	593.0

Generations	Temporal Comparison	N_e(moments)	-95%	+95%	N_e (MLNE)	-95%	+95%
3	1999-2002	244.6	127.2	750.2	496.0	310.1	1015.3
3	2000-2003	474.7	273.6	1017.3	646.1	389.8	1437.3
3	2001-2004	457.2	249.9	1147.0	498.8	303.3	1086.9
3	2002-2005	592.7	393.0	943.0	627.0	457.6	909.2
3	2003-2006	504.3	312.1	920.9	644.2	436.1	1084.1
3	2004-2007	193.7	135.5	281.8	278.6	208.6	390.0
3	2005-2008	335.5	242.3	461.1	402.7	317.7	522.2
3	2006-2009	295.6	218.3	399.9	393.5	312.8	505.7
3	2007-2010	311.9	220.8	448.4	427.3	324.7	587.5
2	1999-2001	116.9	62.0	316.9	269.8	157.3	722.5
2	2000-2002	509.0	287.0	1169.8	717.6	438.5	1562.9
2	2001-2003	450.1	212.5	2351.4	573.8	297.8	2707.0
2	2002-2004	269.5	174.1	449.8	302.0	217.5	459.1
2	2003-2005	592.7	393.8	943.0	855.3	478.2	2549.1
2	2004-2006	305.6	192.5	536.5	536.3	351.0	980.8
2	2005-2007	181.4	128.8	359.1	245.7	192.9	325.0
2	2006-2008	231.6	168.1	320.9	303.3	242.1	391.5
2	2007-2009	276.3	187.6	422.9	417.0	305.3	607.5
2	2008-2010	364.3	252.7	539.2	486.0	361.5	690.4
1	1999-2000	132.7	58.5	1438.5	482.3	225.3	infinity
1	2000-2001	237.9	122.6	738.8	429.9	230.5	2220.7
1	2001-2002	346.5	173.4	1285.5	599.2	343.5	1835.3
1	2002-2003	261.9	153.3	544.8	389.9	264.8	694.7
1	2003-2004	294.0	146.8	1082.7	399.4	146.8	1082.7
1	2004-2005	293.9	165.6	678.1	619.9	358.8	1841.6
1	2005-2006	284.4	184.5	345.2	462.9	332.2	722.5
1	2006-2007	120.3	85.0	172.8	237.6	186.6	319.7
1	2007-2008	163.3	111.5	247.5	316.6	239.9	450.2
1	2008-2009	266.7	182.2	404.1	465.8	347.2	678.9
1	2009-2010	280.7	190.8	430.3	492.1	358.0	744.9

Table 6. Estimates of female effective size and 95% confidence limits. Consecutive estimates are corrected for overlapping generations.

Generations	Temporal Comparison	$Nef_{(moments)}$	-95%	+95%	Nef MLNE	-95%	+95%
12	1987-1999	87.1	11.4	infinity	62.5	19.7	425.26
13	1987-2000	72.3	11.4	384.5	72.8	29.4	219.56
14	1987-2001	359.1	47.6	infinity	878.9	148.6	infinity
15	1987-2002	2057.2	81.0	infinity	449.4	128.7	infinity
16	1987-2003	457.0	52.5	infinity	502.4	128.7	infinity
17	1987-2004	683.6	71.6	infinity	2071.3	206.6	infinity
18	1987-2005	infinity	161.8	infinity	39426.6	377.2	infinity
19	1987-2006	infinity	157.2	infinity	49987.4	421.9	49987.4
20	1987-2007	908.7	90.6	infinity	117.2	232.3	infinity
21	1987-2008	1542.2	128.8	infinity	1222.8	245.0	infinity
22	1987-2009	infinity	214.3	infinity	7501.1	539.8	infinity
23	1987-2010	912.5	92.3	infinity	2801.3	247.3	infinity
11	1999-2010	150.0	27.3	1765.8	653.9	108.2	infinity
10	1999-2009	309.4	53.6	infinity	infinity	335.6	infinity
10	2000-2010	87.7	21.1	244.1	163.6	70.5	434.4
9	1999-2008	302.4	46.1	infinity	45714.5	272.1	infinity
9	2000-2009	144.4	41.4	422.4	440.3	177.1	2400.3
9	2001-2010	1427.6	128.5	infinity	692.0	140.6	infinity
8	1999-2007	243.9	33.8	infinity	20458.9	190.3	infinity
8	2000-2008	129.1	34.4	400.6	436.3	137.0	infinity
8	2001-2009	infinity	215.9	infinity	7575.0	413.4	infinity
8	2002-2010	226.6	56.8	696.1	330.8	132.2	1084.9
7	1999-2006	162.7	26.7	infinity	46261.6	166.9	infinity
7	2000-2007	119.1	27.5	476.6	366.4	118.2	infinity
7	2001-2008	infinity	240.1	infinity	2908.0	222.5	infinity
7	2002-2009	346.5	91.9	1298.2	903.9	318.8	14708.7
7	2003-2010	232.1	50.3	1267	286.8	91.4	2228.6
6	1999-2005	114.0	20.5	infinity	2113.9	109.8	infinity
6	2000-2006	69.1	17.7	200	177.6	78.5	585.81
6	2001-2007	infinity	195.7	infinity	13806.4	216.6	infinity
6	2002-2008	318.2	70.5	4496.5	610.3	179.1	infinity
6	2003-2009	314.6	70.2	4028.2	1080.9	252.2	infinity
6	2004-2010	infinity	566.7	infinity	48746.9	219.8	infinity
5	1999-2004	134.3	19.3	infinity	18119.9	104.0	infinity
5	2000-2005	65.9	16.6	200.7	163.8	70.3	619.21
5	2001-2006	503.8	66.1	infinity	465.3	120.9	infinity
5	2002-2007	229.3	51.4	1539.6	580.3	171.3	infinity
5	2003-2008	265.2	58.8	3747.1	766.8	151.4	infinity
5	2005-2010	677.5	106.0	infinity	618.2	125.6	infinity
4	2004-2009	infinity	162.9	infinity	5387.2	267.5	infinity
4	1999-2003	infinity	27.0	infinity	49340.7	123.4	infinity
4	2000-2004	51.9	12.4	183.9	146.8	56.1	1167.36
4	2001-2005	508.1	52.4	infinity	493.9	101.6	infinity
4	2002-2006	144.1	37.2	490.9	310.1	127.0	1554.27
4	2003-2007	468.9	65.5	infinity	643.8	118.2	infinity
4	2004-2008	infinity	119.5	infinity	1426.3	181.6	infinity
4	2005-2009	infinity	322.2	infinity	48920.7	529.7	infinity
4	2006-2010	569.0	86.0	infinity	611.4	117.0	infinity

Generations	Temporal Comparison	$Nef_{(moments)}$	-95%	+95%	Nef MLNE	-95%	+95%
3	1999-2002	25.5	4.6	131.5	130.1	44.7	infinity
3	2000-2003	61.4	11.8	415.8	190.0	58.9	infinity
3	2001-2004	infinity	56.7	infinity	infinity	104.9	infinity
3	2002-2005	87.1	21.6	276.3	184.5	84.5	641.36
3	2003-2006	113.2	25.5	729.2	253.1	84.1	infinity
3	2004-2007	infinity	139.3	infinity	infinity	138.6	infinity
3	2005-2008	830.9	138.1	infinity	533.2	88.5	infinity
3	2006-2009	infinity	226.0	infinity	49604.8	519.1	49604.8
3	2007-2010	52346.7	92.7	infinity	2782.3	106.2	infinity
2	1999-2001	53.9	7.5	infinity	14699.3	59.3	infinity
2	2000-2002	11.2	2.5	30.3	48.9	29.7	97.9
2	2001-2003	73.6	12.8	infinity	96.3	35.2	infinity
2	2002-2004	59.3	14.3	266.0	192.5	78.2	infinity
2	2003-2005	86.6	17.1	1021.8	207.3	61.5	infinity
2	2004-2006	290.5	34.8	infinity	305.9	79.7	infinity
2	2005-2007	930.5	55.2	infinity	5350.5	124.3	infinity
2	2006-2008	1563.3	128.2	infinity	538.7	69.4	infinity
2	2007-2009	infinity	141.1	infinity	49567.0	301.9	49567.0
2	2008-2010	345.2	54.8	infinity	223.3	63.4	infinity
1	1999-2000	infinity	5.6	infinity	infinity	38.9	infinity
1	2000-2001	24.1	5.1	158.9	218.8	53.9	infinity
1	2001-2002	28.1	6.4	127.6	85.0	44.3	295.8
1	2002-2003	24.0	5.3	86.0	111.0	54.8	637.2
1	2003-2004	156.0	16.9	infinity	39986.0	77.9	infinity
1	2004-2005	677.9	30.5	infinity	436.8	74.4	infinity
1	2005-2006	169.7	28.6	infinity	444.5	112.0	infinity
1	2006-2007	163.1	23.5	infinity	615.3	107.7	infinity
1	2007-2008	infinity	101.6	infinity	63447.5	171.3	63447.5
1	2008-2009	infinity	271.5	infinity	63486.3	337.8	63187.8
1	2009-2010	565.0	54.9	infinity	458.0	109.0	infinity

census size of the population made in October 2009 (Dudley et al. 2010). 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).

In contrast to estimates of effective size made from microsatellite data, those obtained from mitochondrial DNA haplotype frequency data showed a decline in the female effective size for the 2009-2010 comparisons from recent years. In 2010, the N_e estimates obtained from mtDNA and microsatellies were comparable. There are several possible explanations for the discrepancy between some of the mitochondrial and nuclear effective size estimates obtained in previous years including i) unequal sex ratio iii) differences in precision of estimates and iv) the effect of low frequency alleles. For disparities in sex ratio to affect N_e, ten-fold differences are required so this is unlikely to be responsible. Microsatellites have greater power to detect changes in allele frequencies because they are based on nine independent loci (and approximately 261 alleles across all loci) whilst estimates from mitochondrial DNA are based on a single locus (15 different alleles). This difference in power between mitochondrial and nuclear markers may partly explain the disparity. Turner et al. (2001) demonstrated that the temporal method can overestimate N_e in several instances including when i) the proportion of rare alleles in the data set is high such as in microsatellites and ii) when the number of individuals sampled is small. The presence of rare allele is unlikely to explain the disparity because microsatellite (which have more rare alleles) estimates are lower rather than higher than mitochondrial estimates. The maximum likelihood approach is less affected by rare alleles and although these estimates are larger than the temporal method estimates, they are still a fraction of those from mitochondrial DNA.

Several other assumptions are made by the methods used to estimate N_e including that population subdivision and migration does not change gene frequencies within the population over the sampling period. There is no evidence of persistent population structure within the Rio Grande silvery minnow population. Augmentation of the wild population with large numbers of captive bred fish in recent years, may be a proxy for migration. Captive bred fish are derived from a relatively limited number of broodstock that may cause a random divergence of allele frequencies between them and the wild population. Ryman and Laikre (1991) suggested that in some cases supportive breeding may cause a decrease in the effective size of the 'wild' population. It was postulated that this could occur if the effective size of the captive population is

small, but survival of captive fish is higher than for wild fish. Once released, captive fish may therefore comprise a disproportionate component of the population. The greatest risk of this occurring is when the effective size of the wild population is small and the contribution of the captive stock is large but is characterized by small N_e (Ryman and Laikre 1991). The data presented here and in Aló and Turner (2005) indicates that N_e of the wild population in silvery minnow is small and in years where captive stocks are all derived from captive spawning (as opposed to captive reared wild-caught eggs) the captive stock may have smaller values of N_e. We can postulate, that in years where there is poor spawning and poor recruitment in the wild as occurred in 2006, captive fish released the following spring may comprise a disproportionately large fraction of the population. In this scenario, the effective size of the population may be reduced.

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

In 2010 we characterized genetic variation in ten lots of captive spawned fish that were released at four localities in the Big Bend National Park. These fish were bred at Dexter and at the Albuquerque Biological Park. The fish bred at Dexter in 2009 were a mixed lot (CsDx05 and CsDX06) and were produced using group spawning and paired mating. With the exception of allelic richness, 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.

Our results for 2010 are consistent with our previous studies of captive Rio Grande silvery minnow stocks, and suggest that, when possible, wild caught eggs should be salvaged and reared for repatriation to the river and for refreshing captive stocks. Using stocks reared from wild-caught eggs would be particularly beneficial for the Big Bend reintroduction program, as these stocks tend to contain more of the rare alleles present in the wild population. It is also important to maintain as many groups of captive fishes at different rearing and grow-out facilities as practical, as mixed-lot repatriates appear to represent more genetic diversity than single lots, perhaps due to slight variation among rearing conditions and increased numbers of broodstock for mixed lots.

Genetic structure and diversity comparisons between middle Rio Grande reaches

Critical habitat in the Middle Rio Grande is fragmented by four dams, which define three distinct reaches: Angostura, Isleta, and San Acacia. Like previous years (where sufficient data were available to test for genetic differences among reaches) no statistically significant spatial genetic

structure was identified among river reaches in the middle Rio Grande. The finding of no structure among reaches is not surprising as distinct river reaches are connected by substantial gene flow. Gene flow among reaches is facilitated by transport of eggs and larvae and augmentation activities. Prior to fall 2005 all augmentation occurred in the Angostura reach. Since fall 2005, silvery minnow have been stocked in both the Isleta and San Acacia reaches (Remshardt 2007). No stocking has occurred so far in 2010. Interestingly, heterozygosity and microsatellite allelic richness have been generally higher from 2005-2010 than in previous years and have also remained more stable. For mitochondrial DNA data, higher values of gene diversity and allelic richness were recorded for fish collected from the Isleta and San Acacia reaches. Stability of diversity estimates may be a consequence of both stocking of these reaches and less river intermittency during this period.

Acknowledgements

Our sincere thanks are extended to C. S. Altenbach, Terina Perez and staff of the Albuquerque Biological Park, M. Ulibarri and Connie-Keeler Foster and staff (U.S. FWS, Dexter National Fish Hatchery and Technology Center), S. P. Platania (SPP), J. E. Brooks (U.S. FWS), R. K. Dudley, A. M. Snyder (University of New Mexico, Museum of Southwestern Biology), M. D. Porter (Bureau of Reclamation), the Albuquerque Fishery Resources Office of the U.S. Fish and Wildlife Service particularly J. Remshardt, and staff of the Museum of Southwestern Biology for technical and logistic support throughout the project. T. Diver, A. Sharp, S. Netz, T. Max, M. A. Benavides, D. Alò, W. Wilson, G. Moyer, C. Cooper, and M. Foster provided laboratory and/or field assistance. Kevin Buhl provided finclips of captive RGSM (TE046447-0). G. Rosenberg and staff of the UNM Molecular Biology Core Facility provided vital technical support. Funding was provided by U.S. Bureau of Reclamation through the Middle Rio Grande Endangered Species Collaborative Workgroup, U.S. Fish and Wildlife Service, and the National Science Foundation, New Mexico Department of Game and Fish and U. S. Forest Service. Rio Grande silvery minnow were collected under Federal Fish and Wildlife Permits TE038055-0 (TFT) and New Mexico Department of Game and Fish Scientific Collecting Permits 1896 (SPP) and 3015 (TFT). Fin clips were also provided by U.S Fish and Wildlife Service New Mexico Fish Conservation Office.

Literature Cited

Alò, D. & T. F. Turner. 2005. Habitat fragmentation lowers the effective size to census size ratio (N_e/N) in ecological time: a case study of the endangered Rio Grande silvery minnow, *Hybognathus amarus*. *Conservation Biology* 19 (4), 1138-1148.

Avise, J.C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA. (447 pp.).
Bessert, M.L., & G. Ortí. 2003. Microsatellite loci for paternity analysis in the fathead minnow, *Pimephales promelas* (Teleostei: Cyprinidae). *Molecular Ecology Notes* 3, 532-534.
Bestgen K.R. & S.P. Platania. 1991. Status and conservation of the Rio Grande silvery minnow, *Hybognathus amarus*. *Southwestern Naturalist*. 36, 225–232.
Cook, J., Bestgen, K. R., Propst, D. L. & T. L. Yates. 1992. Allozyme divergence and systematics of the Rio Grande silvery minnow, *Hybognathus amarus* (Teleosteri: Cyprinidae). *Copeia* 1998: 36-44.
Dimsoski, P., G. Toth, & M. Bagley. 2000. Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). *Molecular Ecology* 9: 2187-2189.
Dudley, R. K. 2004. *Ichthyofaunal drift in fragmented rivers: empirically-based models and conservation implications*. Ph. D. thesis. Univ. New Mexico, Albuquerque, NM.
Dudley, R. K. and S. P. Platania. 2008. Rio Grande silvery minnow population monitoring program results from December 2006 to October 2007. Final report submitted to US Bureau of Reclamation Albuquerque Area Office 22nd August 2008.
Dudley, R. K. and S. P. Platania. 2008. Summary of Rio Grande silvery minnow population monitoring program results from August 2008. Report submitted to US Bureau of Reclamation Albuquerque Area Office 19th September 2008.
Dudley, R. K. G.C White, S. P. Platania and D.A. Helfrich. 2009. Rio Grande silvery minnow population estimation program results from October 2008. Report submitted to US Bureau of Reclamation Albuquerque Area Office April 10th 2009.
Excoffier, L., P. E. Smouse & J. M. Quattro.1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131, 479-491.
Girard, C. 1856. Researches upon the cyprinoid fishes inhabiting the freshwaters of the United States of America from the Mississippi Valley, from specimens in the Museum of the Smithsonian Institution. Proc. Acad. Nat. Sci., Philidelphia 8, 165 - 213.
Goudet, J. 1995. FSTAT Version 1.2: A computer program to calculate F-statistics. *Journal of Heredity* 86, 485-486.
Guo, S. W. & E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alles. *Biometrics* 48, 361-372.
Hillis, D., Moritz, C. & B. Mable. 1996. *Molecular Systematics*, Sinauer. USA
Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* 46 (2), 477-494.
Leberg, P. L. 2002. Estimating allelic diversity: Effects of sample size and bottlenecks. *Molecular Ecology* 11, 2445-2449.
Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
Nei, M. & F. Tajima. 1981. Genetic drift and estimation of effective population size. *Genetics* 98, 625-640.
Osborne, M.J., Benavides, M.A. & T.F. Turner. 2005. Genetic heterogeneity among pelagic egg samples and variance in reproductive success in an endangered freshwater fish, *Hybognathus amarus* (Cyprinidae). *Environmental Biology of Fishes* 73 (4), 463-472.
Osborne, M.J., Benavides, M.A., Aló, D. & T.F. Turner. 2005. Genetic effects of hatchery propagation and rearing in the endangered Rio Grande silvery minnow. *Reviews in Fisheries Science* 14 (1-2), 127-138.
Osborne, M.J., S.R. Davenport, C.W. Hoagstrom and T.F. Turner. (2010). Genetic effective size tracks abundance in a small-bodied cyprinid, Pecos bluntnose shiner. *Molecular Ecology* Volume 19, Issue 14, 2832–2844.
Palsboll, P.J., Berube M., & F. W.Allendorf. 2007. Identification of management units using population genetic data Trends in Ecology & Evolution 22: 11-16.
Peel, D., Ovenden, J.R. & S.L. Peel 2004. NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries. http://www.dpi.qld.gov.au/fishweb/11637.html.
Petit, R. J., El Mousadik, A., & O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12, 844-855.
Platania, S.P. & C.S. Altenbach. 1998. Reproductive strategies and egg types of seven Rio Grande basin cyprinids. *Copeia*, 3, 559-569.

Remshardt, W.J. 2007. Experimental augmentation and monitoring of Rio Grande silvery minnow in the middle Rio Grande, New Mexico. Annual report submitted to the U.S. Bureau of Reclamation, Albuquerque Area Office, NM.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43, 223-225.

Schneider, S., D. Roessli, & L. Excoffier. 2000. ARLEQUIN: A software program for population genetic analyses. Version 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Swartz, M.K., G. Luikart & R.S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution* 22(1), 25-33.

Swofford, D.L. 2001. PAUP. Phylogenetic analysis using parsimony (*and other methods). Vers. 4 Beta. Sinauer, Sunderland, MA.

Turner, T.F. & Osborne M.J. 2006. Genetic monitoring of the Rio Grande silvery minnow: genetic status of wild and captive stocks in 2007. Annual Report submitted to the U.S. Bureau of Reclamation, Albuquerque Area Office July 2007.

Turner, T.F., Osborne M.J., Moyer G.R., Benavides M.A. & D. Alò. 2006. Life history and environmental variation interact to determine effective population to census size ratio. *P Proceedings Royal Society B - Biological Science*, 273(1605), 3065-3073.

Turner T. F., Wares J. P. & J. R. Gold. 2002 Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics*, 162, 1329-1339.

Turner, T., T. Dowling, R. Broughton & J.R. Gold. 2004. Variable microsatellite markers amplify across divergent lineages of cyprinid fishes (subfamily Leusicinae). *Conservation Genetics*, 279-281.

Van Oosterhout, C., Hutchinson, W.F., Wills, D. P. M. & P. Shipley. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4, 535-538.

Wang, J. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research* 78, 243–257.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121, 379–391.

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