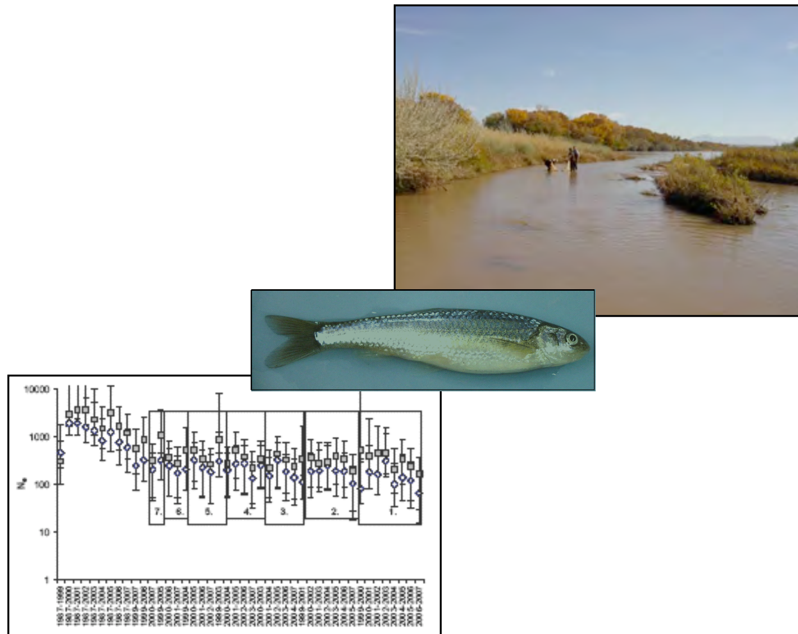
Genetic monitoring of the Rio Grande silvery minnow: Genetic status of wild and captive stocks in 2008.



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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 2008. In 2008 we sampled 479 wild fish, 50 progeny of captive spawning conducted at Dexter National Fish Hatchery and Technology Center, and 100 fish reared from wild-caught eggs collected from the Rio Grande in 2006. These fish are representatives of the fish that were repatriated to the middle Rio Grande in spring and fall 2007.

Major Findings for 2008 are:

(1) Microsatellite gene diversity and heterozygosity increased from values recorded in 2007 but were lower than those observed between 2005 and 2006. Microsatellite allelic richness in 2007 and 2008 was higher than in previous years. Mitochondrial diversity (*h*) of wild Rio Grande silvery minnow was similar to that recorded in 2007 but lower than observed between 2004 and 2006. More mitochondrial haplotypes were identified in 2008 than in prior years. Mitochondrial DNA haplotype frequencies were very similar to 2007 frequencies.

(2) Genetic effective size estimated from mitochondrial DNA haploptye frequencies was higher for the 2007-2008 comparison than for the previous period (2006-2007). The harmonic mean effective size for comparisons from 1999-2008 was 902 (pseudo-maximum likelihood - MLNE) and 339 (temporal) compared to 211 (MLNE) and 62 (temporal) (1999-2007).

(3) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies was lower in 2008 than in 2007. This trend was apparent when either the temporal method or MLNE was used to estimate effective population size.

(4) Captive fish reared from wild-caught eggs (collected in 2006 and released in 2007) had comparable or higher levels of microsatellite diversity and heterozygosity than previous collections of wild-caught eggs. Higher levels of diversity in these wild-caught eggs may be explained by the fact that they are the progeny of fish that spawned in 2005 when the population increased by orders of magnitude. Around 100,000 fish were released in the fall and spring prior to spawning in 2005. In addition, 180,000 captive silvery minnow were released prior to spawning in 2006 and these were genetically quite diverse. Mitochondrial gene diversity was lower however and fewer haplotypes were detected than in stocks reared from eggs collected between 2001 and 2003. Mitochondrial and microsatellite gene diversity was comparable to the source population (wild 2006) whilst allelic richness was marginally lower.

(5) A single lot of progeny from captive spawning was released and therefore genotyped in 2008. Genetic diversity and heterozygosity were comparable to wild stocks but allelic richness was marginally lower. The same trends were apparent for mitochondrial DNA.

(3) In 2008 genetic diversity measures was similar among the three river reaches (Angostura, Isleta and San Acacia). Pairwise F_{STs}, calculated using microsatellite allele frequency data, revealed significant divergence among samples collected from different localities in 2008. Allelic richness increased in all reaches compared to 2007. Heterozygosity decreased in the Angostura reach but increased in both the Isleta and San Acacia reaches.

Introduction

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 focus on genetic analysis of the wild and captive stocks of Rio Grande silvery minnow in 2008. We place these results into context by examining temporal trends in genetic metrics from a dataset that spans more than a decade. 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.

Methods

Sampling- Rio Grande Population

Rio Grande silvery minnows were sampled in the Rio Grande annually from 1999 to 2008 (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

Table 1. Sample sizes, collection localities on the Rio Grande, river reaches and wild 2008 samples.

River Reach	Locality	Sample size
Angostura	Sandia	59
Angostura	Lomitas Negras	42
Angostura	Dixon Rd	4
Angostura	Atrisco	55
Isleta	Alejandro Drain	37
Isleta	Los Lunas Nth Hwy 6	53
Isleta	Below Isleta DD	63
Isleta	Jarales	35
San Acacia	1.5 mile downstream San Acacia Diversion Dam	66
San Acacia	NE Socorro	53

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 24 different captive stocks (five stocks from captive-reared wild caught eggs and 19 stocks from captive spawning) sampled between 2000 and 2006.

In 2008 we screened 479 wild caught Rio Grande silvery minnow and two groups of fish reared in captivity from wild-caught eggs (collected in 2006). We also screened the progeny of captive spawning activities at Dexter that used fish reared from eggs collected from the wild in 2002. Captively reared fish were repatriated to the Rio Grande in spring and fall 2007. There have been no releases of captive Rio Grande silvery minnow in 2008 to date.

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}). 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). 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 2008, 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). Inclusion of age structure does not appreciably alter estimates of N_e for Rio Grande silvery minnow (Turner et al. 2006)

Results

Microsatellites- Genetic Diversity

To date, we have characterized microsatellite diversity in 4146 Rio Grande silvery minnow collected from the wild in 1987 and between 1999 and 2008 and from silvery minnow spawned or reared in captivity and repatriated to the middle Rio Grande. Monitoring of captive stocks has been conducted since the beginning of the augmentation program in 2002. Here we report on data collected in 2008 and compare these to previous data. In 2008, Rio Grande silvery minnow were sampled from 10 localities representing the Angostura, Isleta and San Acacia reaches of the middle Rio Grande (Table 1).

After Bonferroni correction for multiple comparisons there were 130 departures from Hardy-Weinberg equilibrium from a total of 342 comparisons. Forty-seven of these involved wild samples, 23 involved fish reared from wild-caught eggs and 60 involved captive spawned stocks. Among temporal wild samples there was evidence for linkage disequilibrium among two pairs of loci; *Lco3* and *Lco8* and *Lco6* and *Lco7.* Eighteen cases of linkage disequilibrium were detected among captive stocks after Bonferroni correction.

Wild individuals collected in 2008 exhibited similar levels of genetic diversity at nearly every measure compared to wild fishes in 2007 (Table 2, Figure 1). In 2008 observed heterozygosity (H_O) increased slightly to 0.721 from 0.713 in 2007. Allelic richness declined slightly from 14.716 in 2007 to 14.417 in 2008 but both were higher than in 2006 (14.118). Allelic richness for wild fish increased in 2008 in all reaches compared to 2007 values. Heterozygosity declined in the Angostura reach in 2008 but increased in both the Isleta and San

Table 2. Summary statistics for microsatellite and mtDNA – ND4 loci for wild (1987, 1999-2008), 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).

Table 2.

	Microsatellites					Mt-DNA			
Population	**N**	**H_E**	**H_O**	**A_R**	**F_{IS}**	**N**	***h***	**A_R**	**No. Haps**
WILD									
1987	43	0.8184	0.7012	-	0.145	37	0.734	6	6
1999	46	0.8301	0.6187	-	0.258	44	0.427	4.976	5
2000	194	0.8242	0.7140	13.440	0.134	127	0.389	4.968	6
2001	128	0.8284	0.7174	14.094	0.134	121	0.610	8.049	10
2002	390	0.8365	0.6772	13.440	0.191	379	0.630	5.84	8
2003	169	0.8257	0.7272	14.094	0.120	167	0.524	7.106	9
2004	162	0.8477	0.7438	14.281	0.123	164	0.612	8.152	9
2005	394	0.8641	0.7514	13.667	0.131	396	0.610	7.942	10
2006	383	0.8565	0.7521	14.118	0.122	376	0.621	7.664	10
2007	218	0.8529	0.7125	14.716	0.165	218	0.579	7.508	10
2008	479	0.8533	0.7208	14.416	0.155	467	0.572	7.641	11
WILD-CAUGHT EGGS									
WcE-01*	178	0.8472	0.5912	14.647	0.303	157	0.627	6.999	8
WcE-SA-01	50	0.8464	0.7348	13.582	0.133	51	0.624	6	6
WcE-An-02	50	0.8268	0.6856	11.850	0.172	49	0.481	2.949	3
WcE-SA-02	81	0.8565	0.5837	14.352	0.32	81	0.702	7.376	8
WcE-SA-03	51	0.8422	0.7185	14.323	0.148	51	0.714	7.848	8
MJO07-005	54	0.8560	0.7613	13.810	0.112	53	0.602	6.733	7
MJO07-006	49	0.8512	0.7128	13.702	0.164	46	0.581	5.962	6
CAPTIVE SPAWNED									
MJO06-29	50	0.8207	0.7647	10.719	0.069	50	0.517	5	5
Cs-01	64	0.8033	0.6904	12.013	0.142	58	0.46	4.982	5
Cs-An-02	51	0.7104	0.6275	8.270	0.118	51	0	1	1
Cs-SA-02	53	0.8156	0.6626	12.324	0.189	53	0.751	5.919	6
TFT039	51	0.8258	0.6827	12.618	0.175	51	0.558	3.995	4
Cs- 04	50	0.8581	0.7074	14.279	0.177	47	0.586	5.911	6
TFT04-23	50	0.8170	0.7289	11.942	0.109	48	0.593	4.996	5
TFT04-24	48	0.8585	0.7692	12.241	0.105	48	0.609	4.949	5
TFT04-25	50	0.8323	0.8209	11.312	0.014	50	0.702	5.934	6
TFT04-29	54	0.8569	0.7802	14.038	0.09	54	0.609	4.903	5

Table 2. continued

	Microsatellites					Mt-DNA			
Population	**N**	**H_E**	**H_O**	**A_R**	**F_{IS}**	**N**	***h***	**A_R**	**No. Haps**
TFT04-30	56	0.8421	0.7558	13.972	0.103	55	0.656	4.79	5
TFT04-31	50	0.8317	0.7329	12.633	0.12	50	0.706	6.865	7
TFT05-06	50	0.8221	0.6894	9.955	0.163	50	0.625	5.803	6
TFT05-07	49	0.8370	0.7471	12.414	0.109	49	0.55	4.884	5
TFT05-08	50	0.8442	0.7142	11.282	0.155	50	0.611	4.934	5
TFT05-09	50	0.8209	0.7220	12.000	0.122	50	0.506	3.996	4
TFT05-11	51	0.8190	0.7178	11.710	0.125	51	0.573	5.853	6
MJO06-25	50	0.8435	0.7256	13.708	0.141	49	0.635	4.934	5
MJO06-28	50	0.8151	0.6879	11.639	0.158	50	0.738	4.996	5
MJO07-07	50	0.8495	0.7643	12.266	0.101	50	0.605	4.869	5

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**
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	0.629
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	-	-	-	-	-	-	-	-	-
TFT04-23	0.617	0.043	0.191	-		0.043	-	-	-	-	-	0.106	-	-	-
TFT04-24	0.583	0.125	0.208	-	0.021	0.063	-	-	-	-	-	-	-	-	-

	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T
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	-	-	-	-	-	-	-	-	-	-

Acacia reaches (Figure 3). Across years, average diversity measures did not differ significantly between reaches.

Measures of genetic diversity (H_E, A_R, H_O) for captive stocks reared from wild-caught eggs collected in 2006 (MJO07-005 and MJO07-006) were similar to those collected and reared in previous years. In nearly all cases, allelic richness and gene diversity in captively spawned stocks (Figure 2) were lower than in stocks reared from wild-caught eggs. On average, heterozygosity was higher and average inbreeding co-efficients were lower in the captively 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. Allelic richness differed significantly between these groups (P = 0.028) and gene diversity (H_E) approached significance (P = 0.051). Heterozygosity (0.391) and average inbreeding co-efficients (P=0.152) were not significantly different among these groups.

Mt-DNA- Genetic Diversity

Eleven ND4 haplotypes were identified in wild Rio Grande silvery minnow collected in 2008. As observed in previous years, haplotype A was the most frequently encountered (Table 3). In 2008 we detected haplotype P, previously identified in a single fish collected in 2004, and haployepe S (originally were detected in an egg sample collected in 2003) (Osborne et al. 2005). Ten alleles were present in fewer than 15% of individuals. Seven haplotypes were represented in hatchery stocks sampled in 2008 (MJO07-005, MJO07-006, MJO07-007). Gene diversity (h) in wild 2008 samples was lower than in 2001, 2002, 2004, 2005 and 2006 (Figure 1b). Diversity (h) was marginally lower in fish collected from the San Acacia reach than those collected from the Angostura and Isleta reaches in 2008 (Figure 4a-c. Nine haplotypes were detected in both the Angostura and Isleta reaches whilst 10 haplotypes were seen in fish collected in the San Acacia reach. In the Angostura reach gene diversity and haplotype richness increased between 2006 and 2007. Gene diversity ranged from 0.517 to 0.738 among captive stocks sampled in 2006 (Figure 2b).

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 F_{ST} were relatively small but 38 comparisons (out of 55 comparisons) were significant (Table 4a). Pairwise values of F_{ST} were calculated among wild caught, stocks reared from wild caught eggs and captive spawned stocks. The average values of F_{ST} among wild samples was 0.0063, among stocks reared from wild-caught eggs was 0.0232 and for captively spawned stocks F_{ST} = 0.0308. Hierarchical analysis of molecular

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.

4a)

	1987	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
1987	*	0.9990	0.6348	0.6065	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1999	-0.0269	*	0.9990	0.9990	0.9990	0.9990	0.9990	0.9990	0.9990	0.9990	0.0127
2000	0.0000	-0.0218	*	0.0586	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2001	0.0001	-0.0246	0.0016		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2002	0.0063	-0.0289	0.0042	0.0029	*	0.5967	0.0000	0.8633	0.0000	0.0000	0.0000
2003	0.0097	-0.0297	0.0056	0.0050	0.0001		0.0000	0.9482	0.0000	0.0000	0.0000
2004	0.0201	-0.0285	0.0148	0.0142	0.0045	0.0047	*	0.9971	0.5195	0.0000	0.0000
2005	0.0079	-0.0154	0.0074	0.0071	-0.0002	-0.0008	-0.0015		0.0049	0.0000	0.0000
2006	0.0197	-0.0166	0.0173	0.0164	0.0062	0.0042	0.0001	0.0009	*	0.0000	0.0000
2007	0.0210	-0.0202	0.0203	0.0204	0.0108	0.0087	0.0035	0.0018	0.0032		0.0000
2008	0.0453	0.0041	0.0487	0.0484	0.0348	0.0343	0.0217	0.0168	0.0192	0.0156	*

4b)

	1987	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
1987	0.0000	0.0039	0.0000	0.0449	0.4648	0.0225	0.0967	0.1426	0.1494	0.0547	0.0293
1999	0.0759	*	0.5732	0.0791	0.0039	0.4473	0.0811	0.0332	0.0254	0.0752	0.0596
2000	0.1209	-0.0059	*	0.0068	0.0000	0.0098	0.0020	0.0000	0.0000	0.0010	0.0000
2001	0.0239	0.0181	0.0294	*	0.0117	0.0518	0.7412	0.4609	0.2695	0.8525	0.6094
2002	-0.0025	0.0570	0.0808	0.0179	*	0.0010	0.0049	0.0020	0.0088	0.0010	0.0000
2003	0.0373	-0.0019	0.0211	0.0101	0.0286	*	0.2002	0.0127	0.0147	0.0957	0.0440
2004	0.0149	0.0152	0.0342	-0.0034	0.0127	0.0028	*	0.5772	0.4033	0.8291	0.5186
2005	0.0116	0.0239	0.0372	-0.0007	0.0096	0.0094	-0.0013	*	0.4014	0.6875	0.3350
2006	0.0096	0.0254	0.0433	0.0013	0.0075	0.0115	-0.0003	-0.0001	*	0.2725	0.2109
2007	0.0234	0.0152	0.0281	-0.0040	0.0141	0.0049	-0.0033	-0.0016	0.0008	*	0.9502
2008	0.0271	0.0159	0.0288	-0.0017	0.0150	0.0068	-0.0010	0.0002	0.0008	-0.0026	*

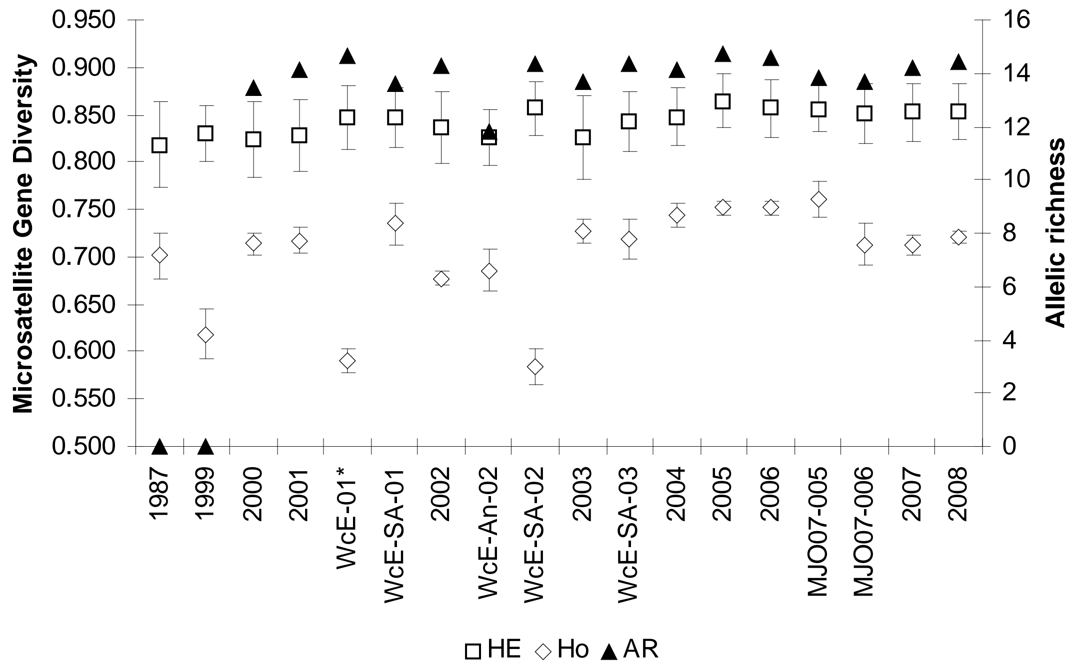
4c)

	Sandia	Lomitas	Atrisco	Isleta DD	Alejandro	Los Lunas	Jareles	DSADD	Socorro
Sandia	*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	0.0010
Lomitas	0.0182	*	0.0010	0.0479	0.0342	0.0039	0.0000	0.0098	0.0537
Atrisco	0.0161	0.0117	*	0.0000	0.0029	0.0371	0.0107	0.0137	0.0898
Isleta DD	0.0089	0.0049	0.0137	*	0.0117	0.0000	0.0000	0.0000	0.0000
Alejandro	0.0181	0.0063	0.0088	0.0077	*	0.0020	0.0000	0.0000	0.0039
Los Lunas	0.0200	0.0093	0.0050	0.0108	0.0095	*	0.0000	0.0000	0.0440
Jareles	0.0117	0.0156	0.0086	0.0120	0.0159	0.0147	*	0.0000	0.0010
DSADD	0.0221	0.0057	0.0051	0.0105	0.0085	0.0080	0.0136	*	0.0000
Socorro	0.0087	0.0050	0.0036	0.0093	0.0091	0.0048	0.0114	0.0075	*

4d)

	Sandia	Lomitas	Atrisco	Isleta DD	Alejandro	Los Lunas	Jareles	DSADD	Socorro
Sandia	*	0.0137	0.0918	0.9766	0.1191	0.1582	0.2383	0.5615	0.6807
Lomitas	0.0487	*	0.4815	0.0147	0.8398	0.5566	0.0508	0.1856	0.0098
Atrisco	0.0206	-0.0049	*	0.1094	0.9522	0.7959	0.0957	0.3828	0.0195
Isleta DD	-0.0142	0.0443	0.0161	*	0.1465	0.1846	0.2051	0.5283	0.5479
Alejandro	0.0198	-0.0177	-0.0188	0.0136	*	0.9551	0.1367	0.5322	0.0596
Los Lunas	0.0119	-0.0066	-0.0118	0.0077	-0.0194	*	0.2363	0.7002	0.1006
Jareles	0.0056	0.0395	0.0281	0.0132	0.0205	0.0070	*	0.4609	0.3604
DSADD	-0.0057	0.0114	-0.0017	-0.0047	-0.0068	-0.0091	-0.0023	*	0.2588
Socorro	-0.0074	0.0581	0.0400	-0.0057	0.0309	0.0197	0.0013	0.0044	*

1a.



1b.

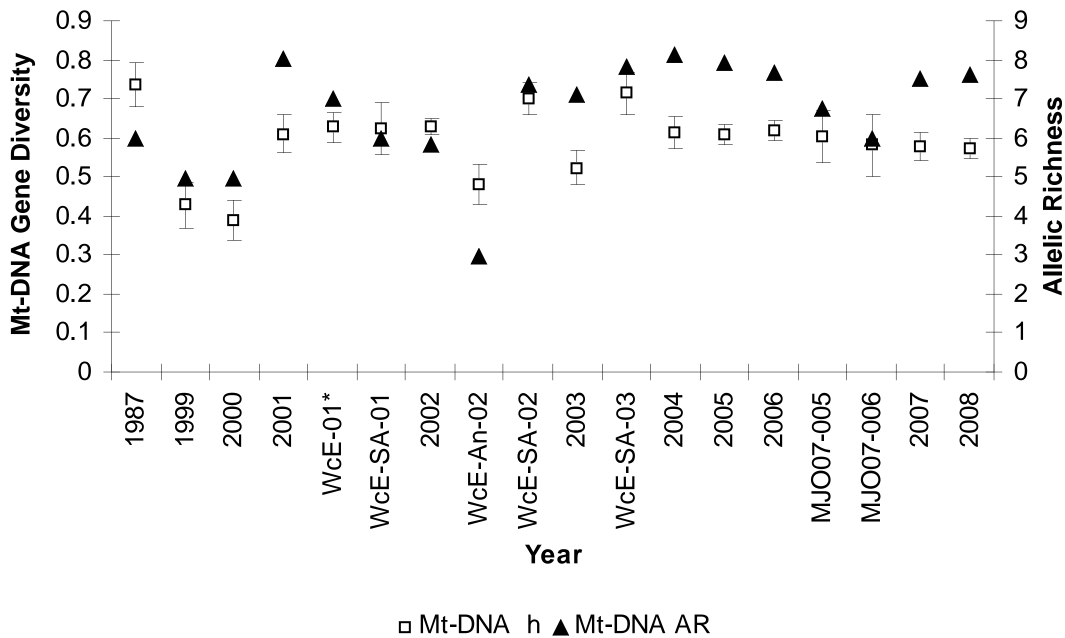
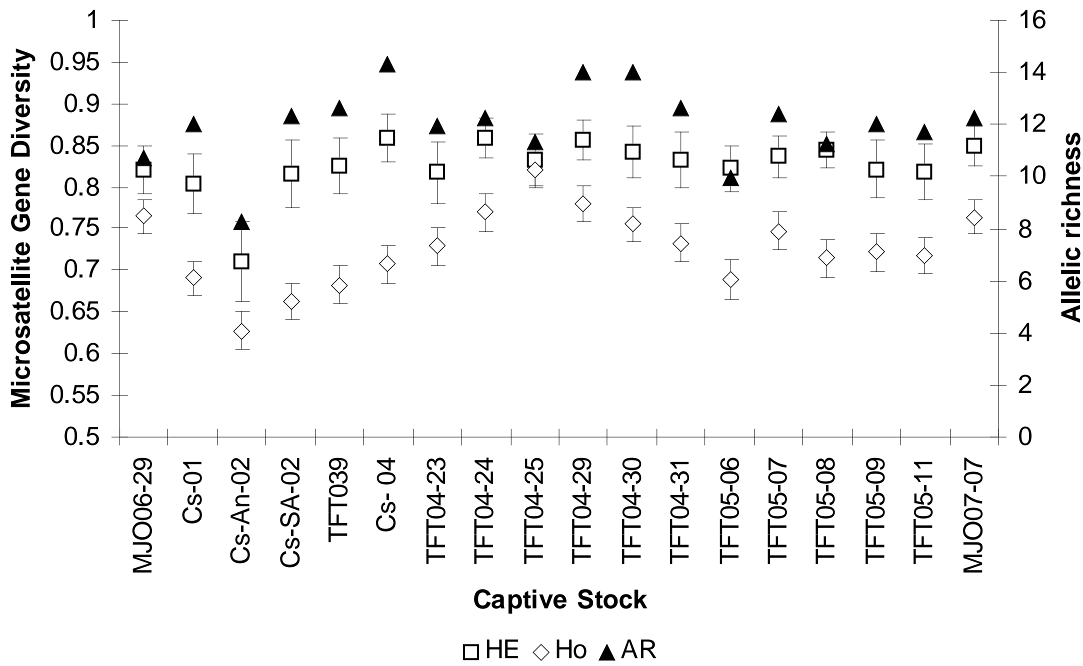


Figure 1. Diversity statistics for wild and stocks reared from wild-caught eggs. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and alleilc richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (h) and haplotype richness are given (**b**). Standard deviation bars are given for h, H_E and H_O.

2a.



2b.

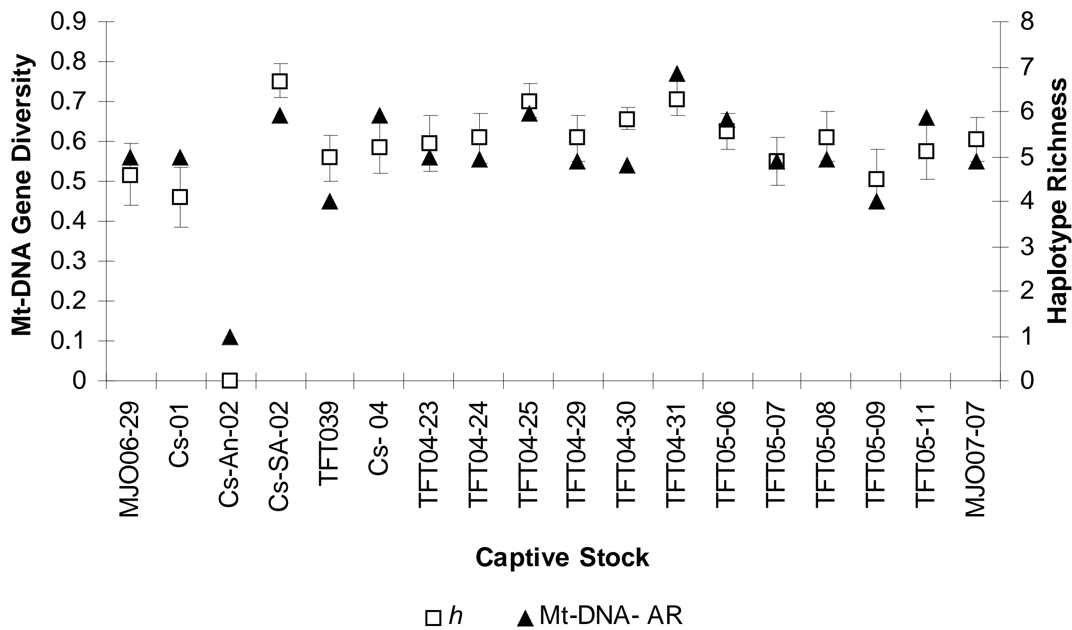
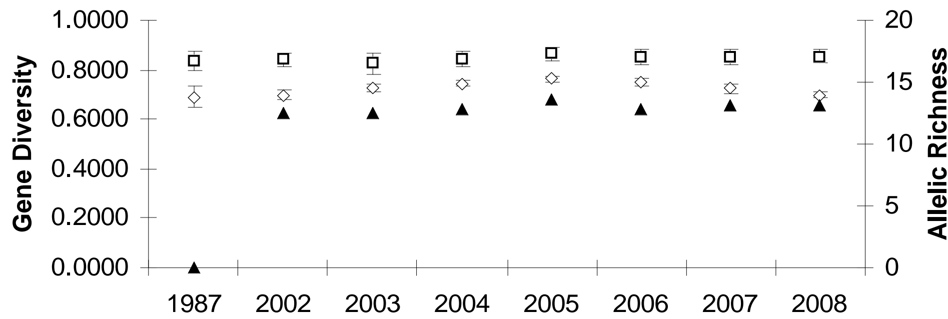


Figure 2. Diversity statistics for captive stocks. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and alleilc richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (*h*) and haplotype richness are given (**b**). Standard deviation bars are given for h, H_E and H_O.

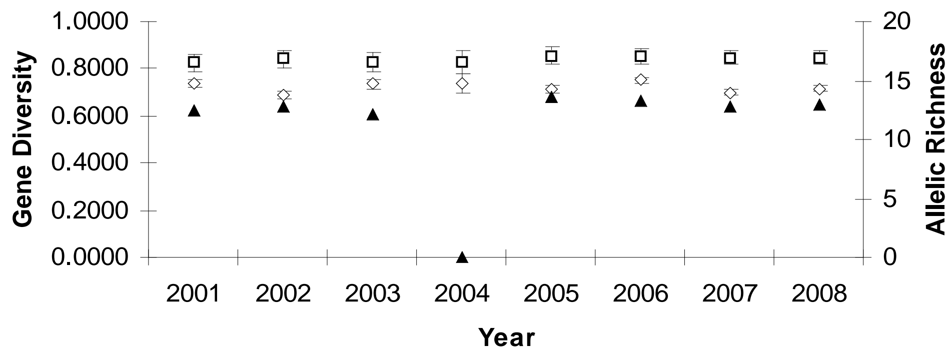
3a.

Angostura



3b.

Isleta



3c.

San Acacia

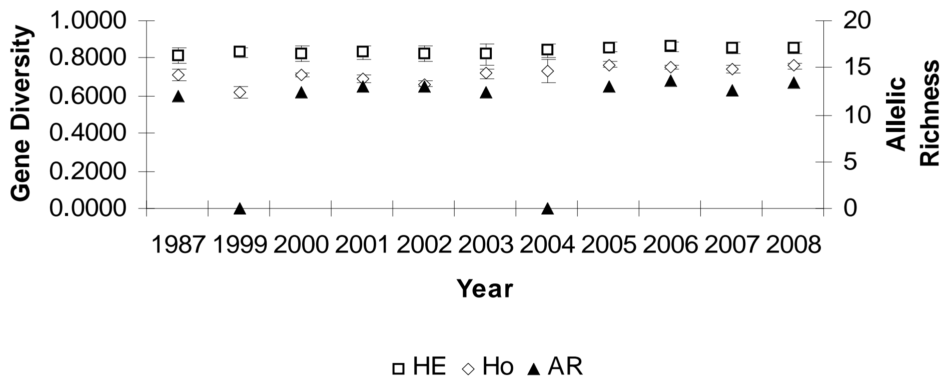
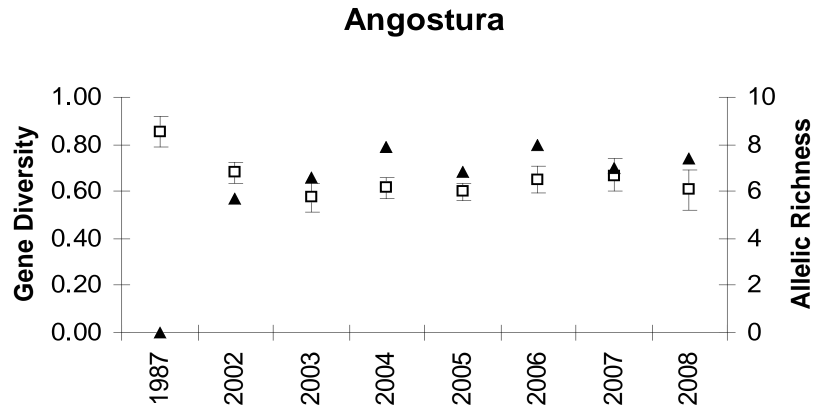
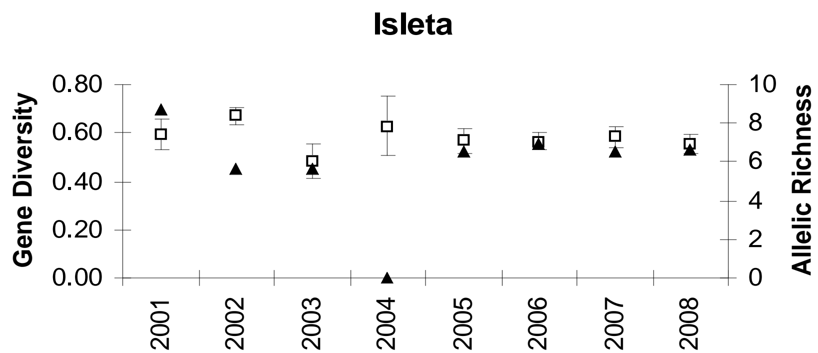


Figure 3. Microsatellite DNA diversity statistics by year and river reach **a**) Angostura, **b**) Isleta and **c**) San Acacia. Gene diversity (*h*) and haplotype richness (A_R) are given. Allelic richness was not calculated for 1987 (Angostura), 1999 (San Acacia), 2004 (Isleta, San Acacia) due to small sample size.

4a.



4b.



4c.

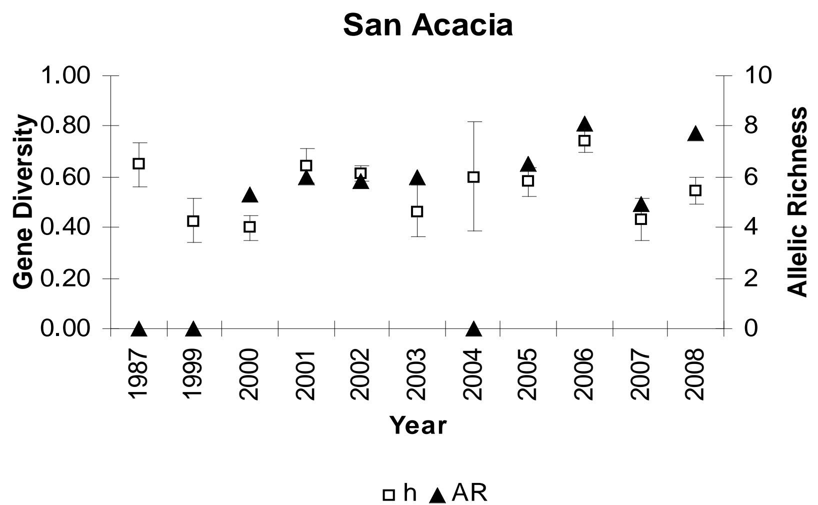


Figure 4. Mitochondrial DNA diversity statistics by year and river reach **a**) Angostura, **b**) Isleta and **c**) San Acacia. Gene diversity (*h*) and haplotype richness (A_R) and given. Allelic richness was not calculated for 1987 (Angostura, San Acacia), 1999, 2004 (Isleta, San Acacia) due to small sample size.

variance (AMOVA) analysis was conducted by grouping wild samples, stocks reared from wild caught eggs and captive spawned stocks. Genetic variation among these samples was small and not significantly different from zero (F_{CT} = 0.001, P=0.1554). A significant portion of genetic variation could be explained by differences among samples within groups (F_{SC} = 0.019, P < 0.0001), and among samples irrespective of their groupings (F_{ST} = 0.020, P < 0.0001). Pairwise F_{STs} were calculated for 2008 among sampling localities. Values of F_{ST} were small yet there were 21 significant values after Bonferroni correction for multiple tests from a total of 36 pairwise comparisons (Table 4c).

Mt-DNA- Population Structure

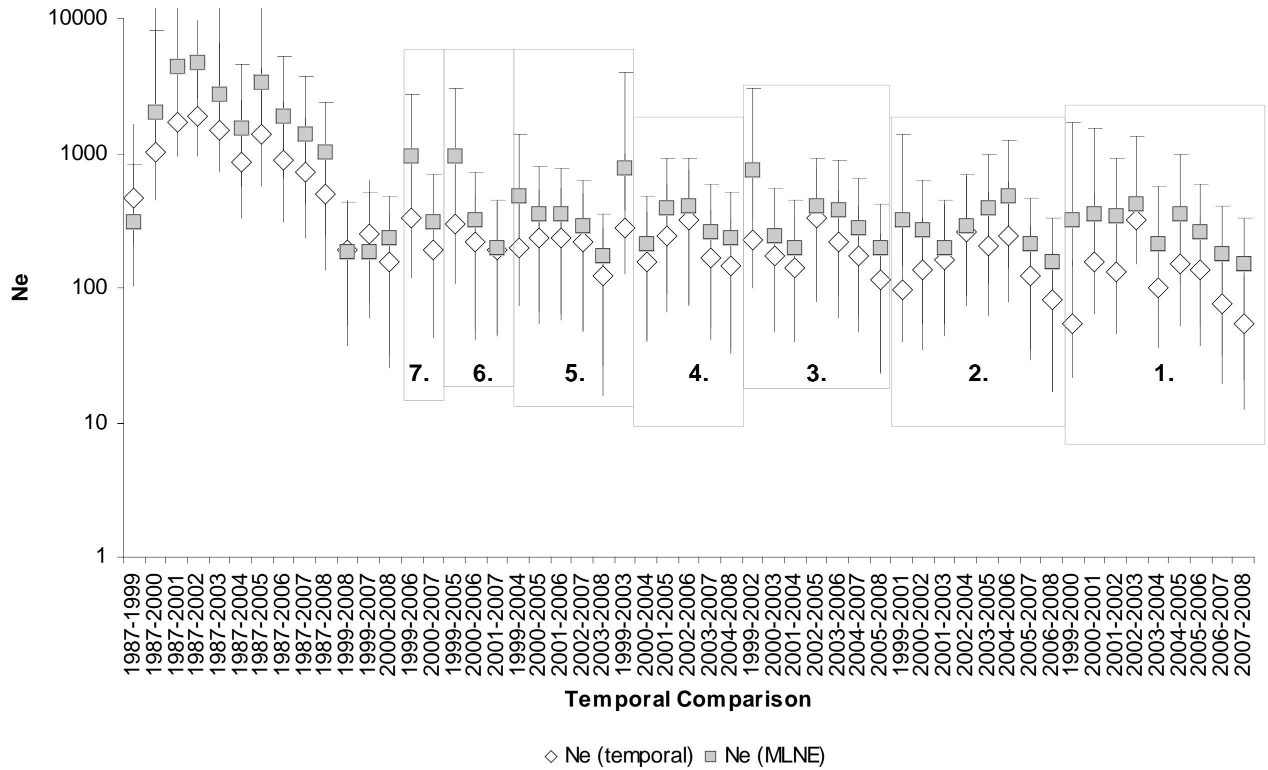
Pairwise F-statistics were calculated between all wild samples collected in 1987 and from 1999-2008. After Bonferroni correction was applied there were six significant comparisons from a total of 45 (Table 4b). Five of the significant comparisons involved the sample collected in 2000 and the remaining significant comparison was between 2002 and 2008. 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) (F_{CT} = 0.0004, P = 0.390) were not significantly different from zero. However, within years a small but significant portion of variation could be partitioned among samples within reaches (F_{SC}=0.0114, P < 0.0001). When samples were grouped by year a small but significant (F_{CT} = 0.0091, P<0.0001) portion of variation could be explained by year. We also calculated pairwise F_{ST} values among 2008 samples collected at different localities. There were no significant differences among localities after Bonferroni correction was applied (Table 4).

Effective Population Size

Variance effective size was estimated between all wild samples collected from 1999 to 2008 (Figure 5a). Ten temporal comparisons were also made with a sample collected in 1987, for all of these N_{eV} was above 450 and five estimates were above 1000. With the exception of the 1987 to 1999 comparison maximum likelihood estimates (MLNE) were larger and all were in excess of 1000. N_{eV} was 54.4 (95 % CIs 41.9 – 70.3) for most recent temporal comparison (2007 and 2008); the lowest estimate seen since genetic monitoring began. The maximum likelihood estimate for the same period was higher (N_e = 150.8, 95 % CIs 130.2 – 177.5) but also the lowest recorded to date. The harmonic mean across all pairwise estimates (1999-2008) was 277 (MLNE) and 153 (N_{eV}).

Trends in variance female effective size (calculated from Mt-DNA haplotype frequencies) showed an increase with an estimate of infinity for 2007 and 2008 comparison and N_{eV} of 538

5a.



5b.

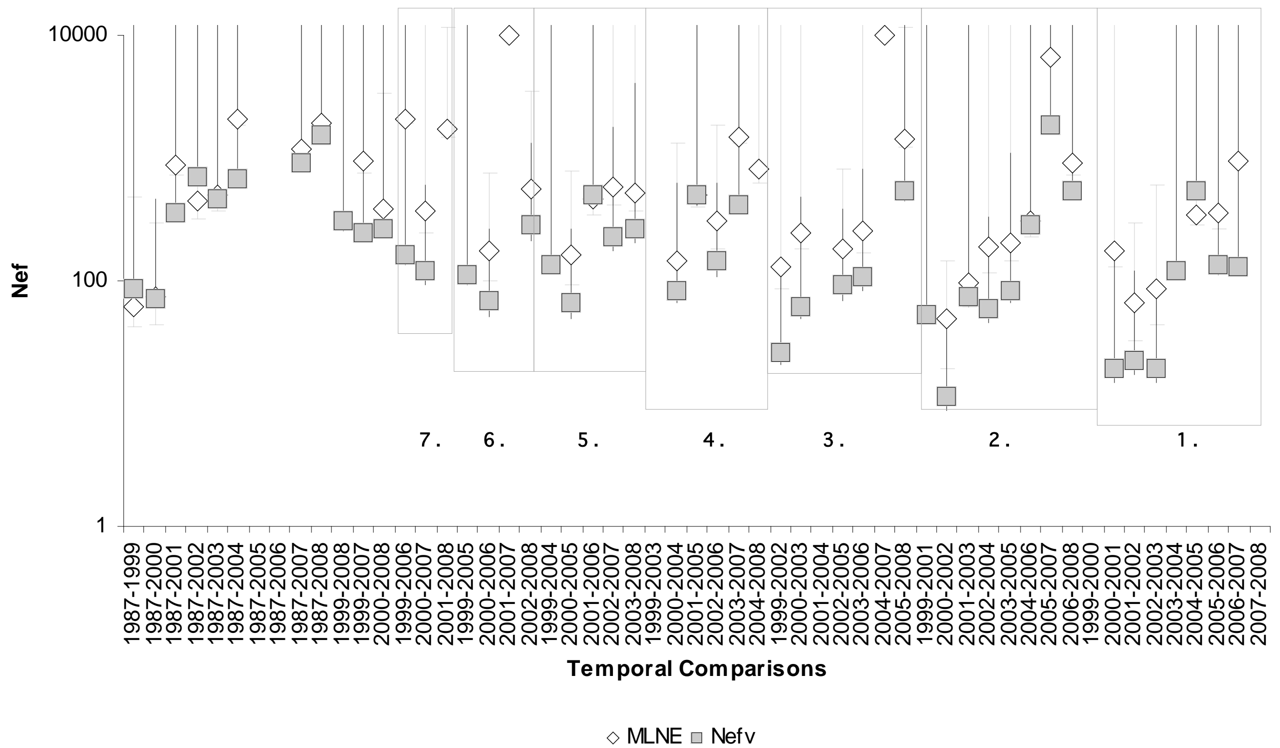


Figure 5. a) Pairwise genetic effective size estimates based on microsatellite DNA (boxes indicate generation intervals separating pairwise comparisons) and **b)** mitochondrial DNA data.

and 927 (MLNE) for the 2006-2008 comparison (Figure 5b). The harmonic mean for samples collected in from 1999 to 2008 was N_{ef} = 258.29 (MLNE) and N_{ef} = 71.33 (NeEstimator) (samples where N_e was infinity were excluded).

Discussion

Genetic status of the species in 2008

To interpret genetic data it is important to consider the demographic trends in the population. Although catch rates for Rio Grande silvery minnow were high in 2008, population densities in previous years also influence the amount of diversity. Favorable spring flows in 2007 resulted in an almost an order of magnitude increase in adult silvery minnow density in the middle Rio Grande in 2007 over 2006 densities; a year of poor spawning and recruitment (Dudley and Platania 2007). Population monitoring data for 2008 shows that in the month immediately preceding spawning, Rio Grande silvery minnow was more abundant than in 2007 (Dudley and Platania 2008). Genetic diversity measures including heterozygosity and average gene diversity increased above those recorded in 2007 in the wild Rio Grande silvery minnow population yet these measures were still lower than those observed between 2004 and 2006. Microsatellite allelic richness in 2007 and 2008 was higher than in previous years. Average inbreeding co-efficients (F_{IS}, which measures the departure of observed and Hardy-Weinberg expected heterozygosity) decreased slightly from 2007. Levels of gene diversity (*h*) at the mitochondrial ND4 of wild Rio Grande silvery minnow was similar to that recorded in 2007 but also lower than observed between 2004 and 2006. More mitochondrial haplotypes were identified in 2008 than in prior years, but this measure is strongly sample-size dependent and 2008 marked the largest sample sizes ever obtained over the history of the study. 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'). Genetic diversity can only be generated by mutation (which is not expected to be a factor over the short time-frame of this study) or by input from genetically diverse captive stocks. For this reason, it take sometime to recover after a year of poor recruitment as occurred in 2006 which presumably resulted in a comparatively few spawners in 2007.

Estimates (calculated from microsatellite data) of variance effective population size (N_{eV}) for 2007-2008 calculated using the temporal and pseudo-maximum likelihood methods, were the lowest observed since genetic monitoring began. 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 greater when spring flows are higher (Dudley 2004).

In contrast to estimates of effective size from microsatellites, those obtained from mitochondrial DNA haplotype frequency data showed a dramatic increase in the female effective size over previous years. There are several possible explanations for discrepancy between mitochondrial and nuclear effective size estimates including i) unequal sex ratio or effective sex ratio, iii) differences in precision of estimates and iv) the effect of low frequency alleles. Disparities in sex ratio are unlikely to be a problem given the degree of skew required for this to affect N_e. However, it would be useful to obtain sex ratio estimates for Rio Grande silvery minnow to examine this in the future. 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) whereas estimates from mitochondrial DNA are based on a single locus (14 different alleles). 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. Maximum-likelihood estimates are less impacted by high incidence of rare alleles (Wang 2001) and although these estimates are larger than the temporal method estimates, they are still a fraction of those from mitochondrial DNA. The presence of rare allele is unlikely to explain the disparity because the microsatellite estimates (which have more rare alleles) are lower rather than higher than the mitochondrial estimates. Additionally, if estimation bias between mitochondrial and microsatellite DNA was responsible for the disparity, we would expect to see this across years not just in 2008.

Several other assumptions are made by the methods used to estimate N_e including population subdivision and migration, do 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 somewhat 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 can cause the effective size of the ‘wild’ population to decrease. 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 postulate, that in years where there is poor spawning and poor recruitment in the wild as occurred in 2006, captive fish released in the fall and following spring, may comprise a disproportionately large fraction of the population. In this scenario the effective size of the population may be reduced. In 2007 three lots of fish were released, one was captive spawned and two were from wild-caught eggs reared in captivity. These eggs were collected in 2006 when spawning and recruitment were poor due to low spring runoff and river drying in the summer. We propose to explore the interaction between the wild population and captive stocks in the future because this interaction, in addition to environmental conditions, clearly impact diversity and effective population size of the wild population of Rio Grande silvery minnow.

Genetic Diversity of Captive Stocks

In 2008 we characterized genetic variation in the sole lot of captive spawned fish that was repatriated to the river in fall 2007. These fish were bred at Dexter National Fish Hatchery and Technology Center using broodstock collected as eggs from the Rio Grande in 2002. Diversity measures for this lot (MJO07-007) were comparable to the wild population. In addition, two lots of fish reared from wild-caught eggs (collected in 2006) were repatriated to the Rio Grande in fall 2007. Diversity measures calculated from microsatellite DNA data were comparable to the wild population and to previous egg collections. There is more variation in diversity measures across all captive spawned stocks and on average, allelic richness is lower than for wild-caught eggs. The diversity of these stocks ultimately depends on the size and diversity of the broodstock. In 2004 hatchery managers began using paired matings and where group spawns were conducted, the numbers of male and female broodstock were equalized. The purpose of theis strategy has been to maximize the genetic diversity and effective size of the captive stocks. 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 (especially allelic richness) than single lots, perhaps due to slight variation among rearing conditions and increased numbers of broodstock for mixed lots. We also suggest that, when possible, wild caught eggs should be salvaged and reared for repatriation to the river and for refreshing captive stocks.

Genetic structure and diversity comparisons between middle Rio Grande reaches

Critical habitat in the Middle Rio Grande is fragmented by four dams that define three distinct reaches: Angostura, Isleta, and San Acacia. Like previous years (where sufficient data were available to test for genetic differences among reaches) we identified no statistically significant spatial genetic structure from microsatellite data among river reaches in the middle Rio Grande. The finding of no significant 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 (from upstream to downstream), augmentation activities, and movement of salvaged fish (from downstream to upstream). Prior to fall 2005 all augmentation occurred in the Angostura reach. Since fall 2005, silvery minnow have also been stocked in both the Isleta and San Acacia reaches (Remshardt 2007). Interestingly, heterozygosity and microsatellite allelic richness have been higher from 2005-2008 than in previous years and have also remained more stable. This is likely a consequence of stocking in downstream reaches, less river intermittency in these reaches during this period, or a combination of both these factors.

As in previous years we identified significant local genetic structuring (based on microsatellite data) of Rio Grande silvery minnow among 2008 collection sites. Local genetic structure is apparent because adults form aggregations that comprise a small yet presumably random subset of the total adult population. This results in a natural sampling effect, so that each aggregation comprises a subset of the total available genetic diversity in a reach. These groups are transitory in nature and therefore do not represent ‘distinct population segments’. The existence of these genetically distinct groups suggests however, that collection of fish for broodstock or for egg collections for hatchery rearing purposes should be from as many localities as possible to account for the presence of local population structure. Collections should be made throughout the current distribution of the species to maximize levels of diversity in hatchery stocks.

Conclusions

We have been monitoring changes in commonly used metrics of genetic diversity such as allelic richness, heterozygosity, and genetically effective population size (N_e) in the middle Rio Grande population of Rio Grande silvery minnow for a decade. The increased abundance in 2007-2008 compared to 2006 has been accompanied by positive changes in diversity measures. Allelic richness (mitochondrial DNA) and microsatellite gene diversity and heterozygosity increased in 2008. In addition the value of the average inbreeding co-efficient declined from 2007 values. There were increases in allelic richness (microsatellites and mitochondrial DNA) in all river reaches and heterozygosity increased from 2007 levels in the Isleta and San Acacia reaches

but declined in the Angostura reach. Mitochdondrial gene diversity also increased in the San Acacia reach, but remained similar to 2007 levels in the Angostura and Isleta reaches. Increases in gene diversity reflect increases in relative frequencies of previously rare genetic variants and not from new mutations in the population. Mitochondrial DNA data indicated a sharp increase in the genetic effective size for the most recent temporal comparison (2007-2008). These positive developments are likely a result of increases in abundance in the wild in 2007-2008, decreased severity of river drying, and inputs of diversity from captive stocks. In contrast to these findings, the genetic effective size estimates calculated from microsatellites are small, as previously recorded. This may be caused by the interaction of captive and wild stocks and their genetic effective sizes. How this interaction affects effective size is likely determined partly by the extent and magnitude of spring flows as well as the degree of summer drying as discussed above. Low effective size of microsatellites suggests that demographic dynamics in the wild may still exert downward effects on total genetic diversity in the future, particularly in the absence of reconnection of river reaches and stocking.

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