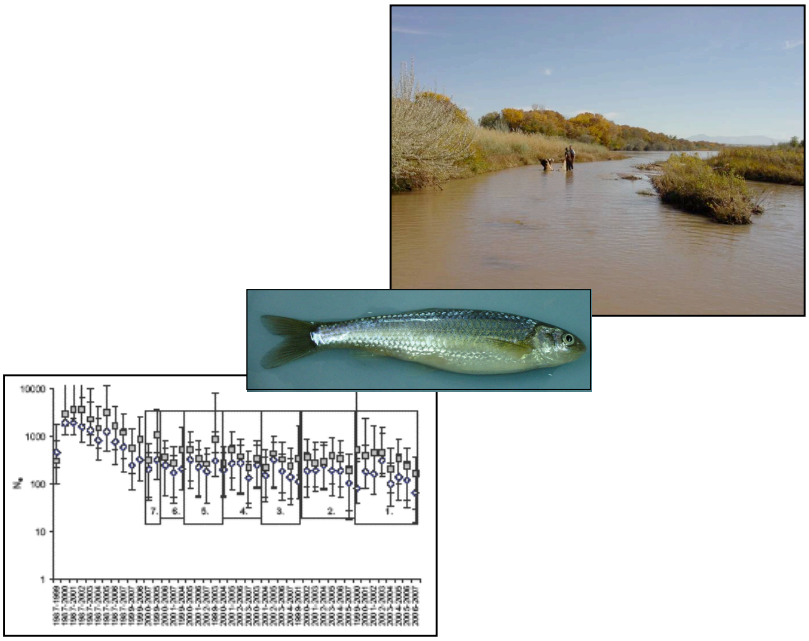
Genetic monitoring of the Rio Grande silvery minnow: Genetic status of wild and captive stocks in 2009.



Annual report FY 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 2009. In 2009 we sampled 478 wild fish and 531 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 December 2008.

Major Findings for 2009 are:

(1) Microsatellite gene diversity increased in 2009 from values recorded in 2008 and gene diversity was the highest recorded since genetic monitoring commenced in 1999. In contrast, heterozygosity has declined consistently since 2004. Allelic richness has remained relatively stable since 2006. Mitochondrial gene diversity was similar to 2008 values whilst haplotype richness increased from 2005-2008 values. Mitochondrial DNA haplotype frequencies were very similar to 2008 frequencies.

(2) Genetic effective size estimates from mitochondrial DNA haploptye frequencies for 2008-2009 were very similar to the 2007-2008 comparison. These N_{ef} estimates were considerably higher than recorded for the previous period (2006-2007). The harmonic mean effective size for all pairwise comparisons from 1999-2009 was 77.32 (MLNE) and 288.65 (moments).

(3) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies was higher for the 2008-2009 (Moments N_{eV}=210.1, MLNE N_{eV}=367.3) temporal comparison than for the previous period 2007-2008 (130, 259). 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 but fewer haplotypes were detected in each of the captive stocks. However, across all captive stocks, a similar number of mitochondrial DNA haplotypes were detected to the wild population.

(5) In 2009 mitochondrial DNA gene diversity and allelic richness increased in a downstream direction (i.e fish from the San Acacia reach had the highest diversity). For microsatellite data there was not a clear directional trend in diversity measures.

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 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. 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 2009 (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 2009 were released at four localities (Rio Grande Village, Adams Ranch, Grassy Banks, Santa Elena Canyon) in the Big Bend National Park, Texas. These are samples MJO08-006, MJO08-007, MJO08-008, MJO08-009 and MJO09-001, MJO09-002, MJO09-003, MJO09-004.

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*]

Table 1. Sample sizes, collection localities on the Rio Grande, river reaches and wild 2009 samples.

River Reach	Locality	Sample Size
Angostura	Rio Rancho	1
Angostura	Sandia	50
Angostura	AMAFCA Channel	23
Angostura	Lomitas Negras	1
Angostura	Dixon Rd	49
Angostura	Central Ave Bridge	50
Angostura	Atrisco	2
Isleta	Below Isleta DD	52
Isleta	Alejandro Drain	51
Isleta	Los Lunas	50
San Acacia	San Acacia Diversion Dam	50
San Acacia	San Antonio	50
San Acacia	San Marcial	50

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).

Results

Microsatellites- Genetic Diversity

To date, we have characterized microsatellite diversity in 4146 Rio Grande silvery minnow collected from the wild in 1987 and annually between 1999 and 2009 and from silvery minnow spawned and/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 2009 and compare these to previous data. In 2009, Rio Grande silvery minnow were sampled from 13 localities representing the Angostura, Isleta and San Acacia reaches of the middle Rio Grande (Table 1).

After Bonferroni correction for multiple comparisons there were 131 departures from Hardy-Weinberg equilibrium from a total of 423 comparisons. Forty-three of these involved wild samples, 18 involved fish reared from wild-caught eggs and 70 involved captive spawned stocks. Among temporal wild samples there was evidence for linkage disequilibrium among four pairs of loci; *Lco3* /*Lco6*, *Lco6* /*Lco7, Lco7/Ca6 and Ca6/Ppro118*. Eighteen cases of linkage disequilibrium were detected among captive stocks.

Wild individuals collected in 2009 exhibited similar levels of genetic diversity at nearly every measure compared to wild fishes in 2008 (Table 2, Figure 1). In 2009, observed heterozygosity (H_O) decreased slightly to 0.6896 from values recorded from 2003-2008. Allelic richness was comparable to values seen in 2008 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. 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 = 0.016) and F_{ST} (P = 0.047) differed significantly between these groups whilst gene diversity (P = 0.417), heterozygosity (P = 0.467) and average inbreeding co-efficients (P = 0.271) however were not significantly different among groups. Allelic richness declined in 2009 in the Angostura reach compared to 2007 and 2008 values whist A_R was similar among years both the Isleta and San Acacia reaches. Heterozygosity declined all reaches in 2009 compared to values recorded in 2008 (Figure 2a-c).

Mt-DNA- Genetic Diversity

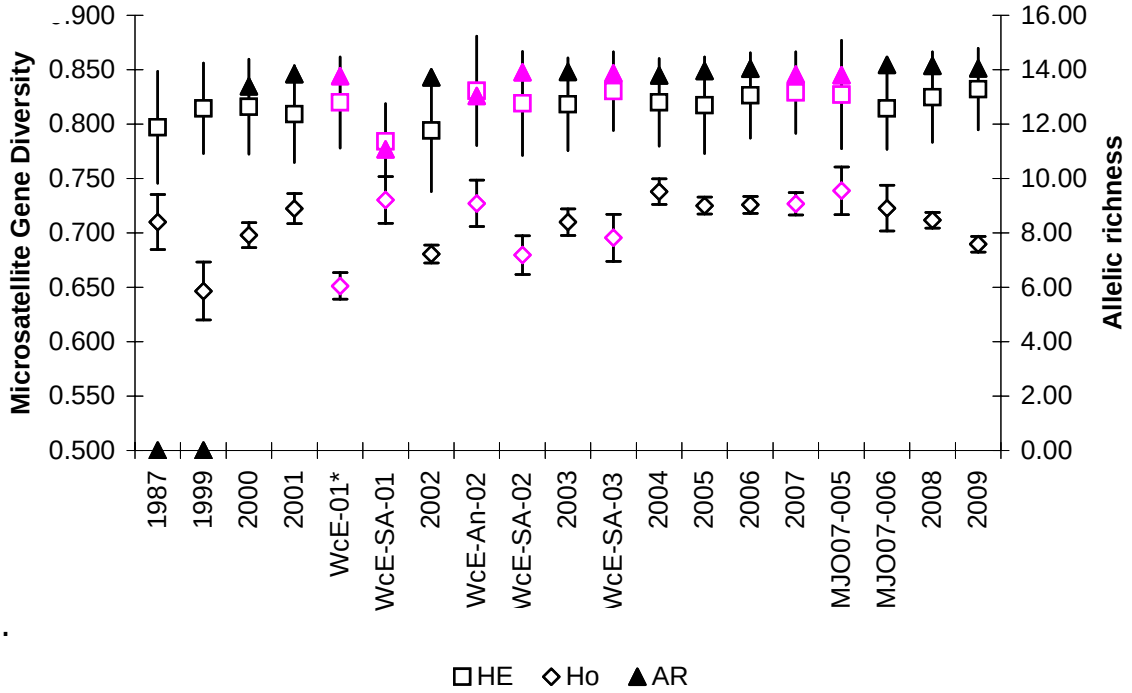
Twelve ND4 haplotypes were identified in wild Rio Grande silvery minnow collected in 2009. As observed in previous years, haplotype A was the most frequently encountered (Table 3). Ten haplotypes were present in fewer than 10% of individuals. Gene diversity (*h*) in wild 2009 samples was lower than in 1987, 2001, 2002, 2004-2007 (Table 2, Figure 1b). In 2009 diversity (*h*) was marginally lower in fish collected from the Angostura reach than those collected from the Isleta and San Acacia reaches. Twelve haplotypes were detected in the Angostura reach whilst eight and nine haplotypes were seen in fish collected in the Isleta and San Acacia reaches respectively. Allelic richness was higher in both the Isleta and San Acacia reaches. In the Angostura reach gene diversity decreased in 2009 compared to values recorded between 2004 and 2008 whilst it increased in the Isleta and San Acacia reaches from 2005-2008 and 2007-2008 respectively (Figure 4a-c).

Table 2. Summary statistics for microsatellite and mtDNA – ND4 loci for wild (1987, 1999-2009), hatchery reared wild-caught eggs, 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), haplotype richness (A_R) and observed number of haplotypes are given.

		usats				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	0.816	0.698	0.145	13.393	127	0.389	4.968	6
2001	128	0.809	0.722	0.108	13.836	121	0.610	8.049	10
2002	389	0.794	0.681	0.143	13.717	379	0.630	5.840	8
2003	169	0.818	0.710	0.133	13.921	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	0.825	0.712	0.137	14.139	467	0.572	7.641	11
2009	478	0.832	0.690	0.171	14.046	465	0.578	8.070	12
Mean		**0.817**	**0.707**	**0.135**	**13.865**		**0.574**	**6.993**	**8.833**
WILD-CAUGHT EGGS									
WcE-01*	178	0.820	0.651	0.206	13.766	157	0.627	6.999	8
WcE-SA-01	50	0.784	0.730	0.070	11.065	51	0.624	6.000	6
WcE-An-02	50	0.831	0.727	0.126	13.038	49	0.481	2.949	3
WcE-SA-02	81	0.819	0.680	0.171	13.907	81	0.702	7.376	8
WcE-SA-03	51	0.830	0.696	0.164	13.868	51	0.714	7.848	8
MJO07-005	54	0.827	0.739	0.108	13.801	53	0.602	6.733	7
MJO07-006	49	0.814	0.723	0.114	14.171	46	0.581	5.962	6
Mean		**0.818**	**0.706**	**0.137**	**13.374**		**0.619**	**6.267**	**6.571**
CAPTIVE SPAWNED									
MJO06-29	50	0.804	0.745	0.074	10.394	50	0.517	5.000	5
Cs-01	64	0.794	0.659	0.172	11.931	58	0.460	4.982	5
Cs-An-02	51	0.686	0.675	0.015	7.507	51	0.000	1.000	1
Cs-SA-02	53	0.803	0.673	0.163	12.034	53	0.751	5.919	6
TFT039	51	0.806	0.700	0.133	11.691	51	0.558	3.995	4
Cs- 04	50	0.824	0.691	0.163	13.247	47	0.586	5.911	6
TFT04-23	50	0.779	0.683	0.124	11.071	48	0.593	4.996	5
TFT04-24	48	0.828	0.717	0.135	11.087	48	0.609	4.949	5

Population	N	H_E	H_O	F_{IS}	A_R	N	*h*	A_R	No Haps
TFT04-25	50	0.810	0.768	0.053	10.661	50	0.702	5.934	6
TFT04-29	54	0.839	0.763	0.092	13.028	54	0.609	4.903	5
TFT04-30	56	0.826	0.727	0.121	13.524	55	0.656	4.790	5
TFT04-31	50	0.805	0.701	0.130	11.998	50	0.706	6.865	7
TFT05-06	50	0.792	0.649	0.183	9.768	50	0.625	5.803	6
TFT05-07	49	0.797	0.704	0.117	11.305	49	0.550	4.884	5
TFT05-08	50	0.804	0.663	0.178	10.584	50	0.611	4.934	5
TFT05-09	50	0.804	0.717	0.109	11.899	50	0.506	3.996	4
TFT05-11	51	0.808	0.692	0.144	11.447	51	0.573	5.853	6
MJO06-25	50	0.814	0.721	0.115	13.282	49	0.635	4.934	5
MJO06-28	50	0.805	0.705	0.125	11.295	50	0.738	4.996	5
MJO07-07	50	0.813	0.739	0.091	11.992	50	0.605	4.869	5
MJO08_06	50	0.827	0.669	0.192	13.493	45	0.664	6.939	7
MJO08_07	50	0.841	0.721	0.144	13.105	50	0.625	6.803	7
MJO08_08	50	0.835	0.711	0.150	13.487	49	0.706	5.997	6
MJO08_09	51	0.843	0.715	0.153	13.849	51	0.658	5.995	6
MJO09_01	68	0.818	0.706	0.138	13.606	62	0.594	8.329	9
MJO09_02	72	0.799	0.670	0.162	13.354	68	0.540	6.431	7
MJO09_03	71	0.811	0.719	0.115	13.120	64	0.619	5.818	6
MJO09_04	69	0.817	0.713	0.128	13.228	64	0.436	5.595	6
Mean		**0.808**	**0.704**	**0.129**	**12.035**		**0.587**	**5.408**	**5.536**

1a.



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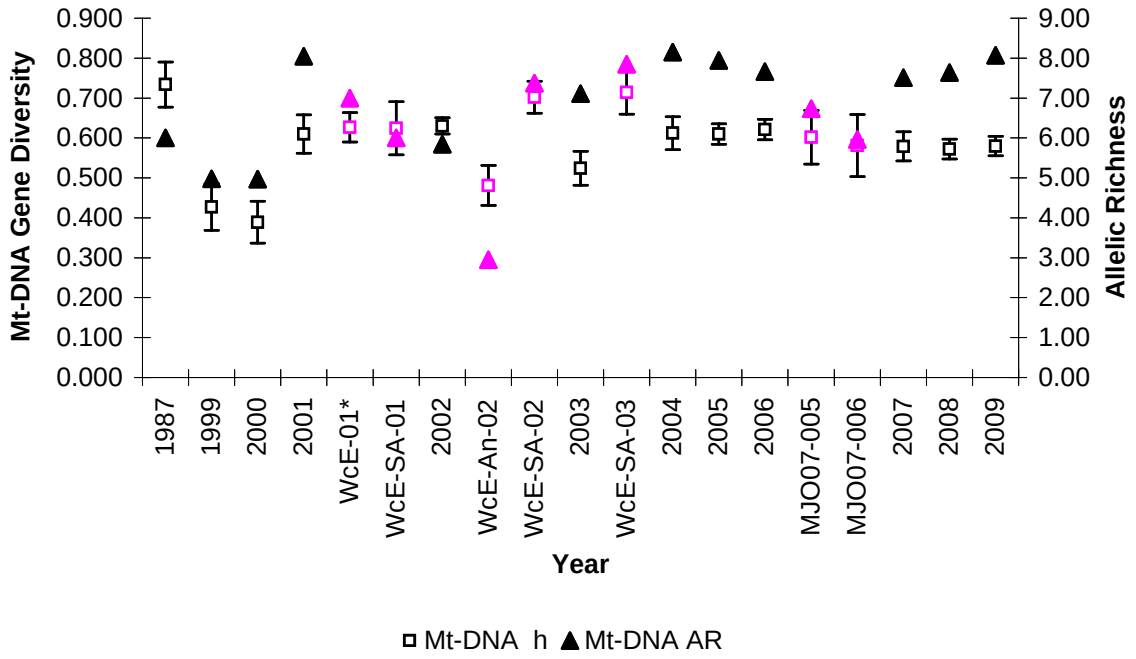


Figure 1. Diversity statistics for wild and stcoks reared from wild-caught eggs. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (*h*) and haplotype richness are given (**b**). Standard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

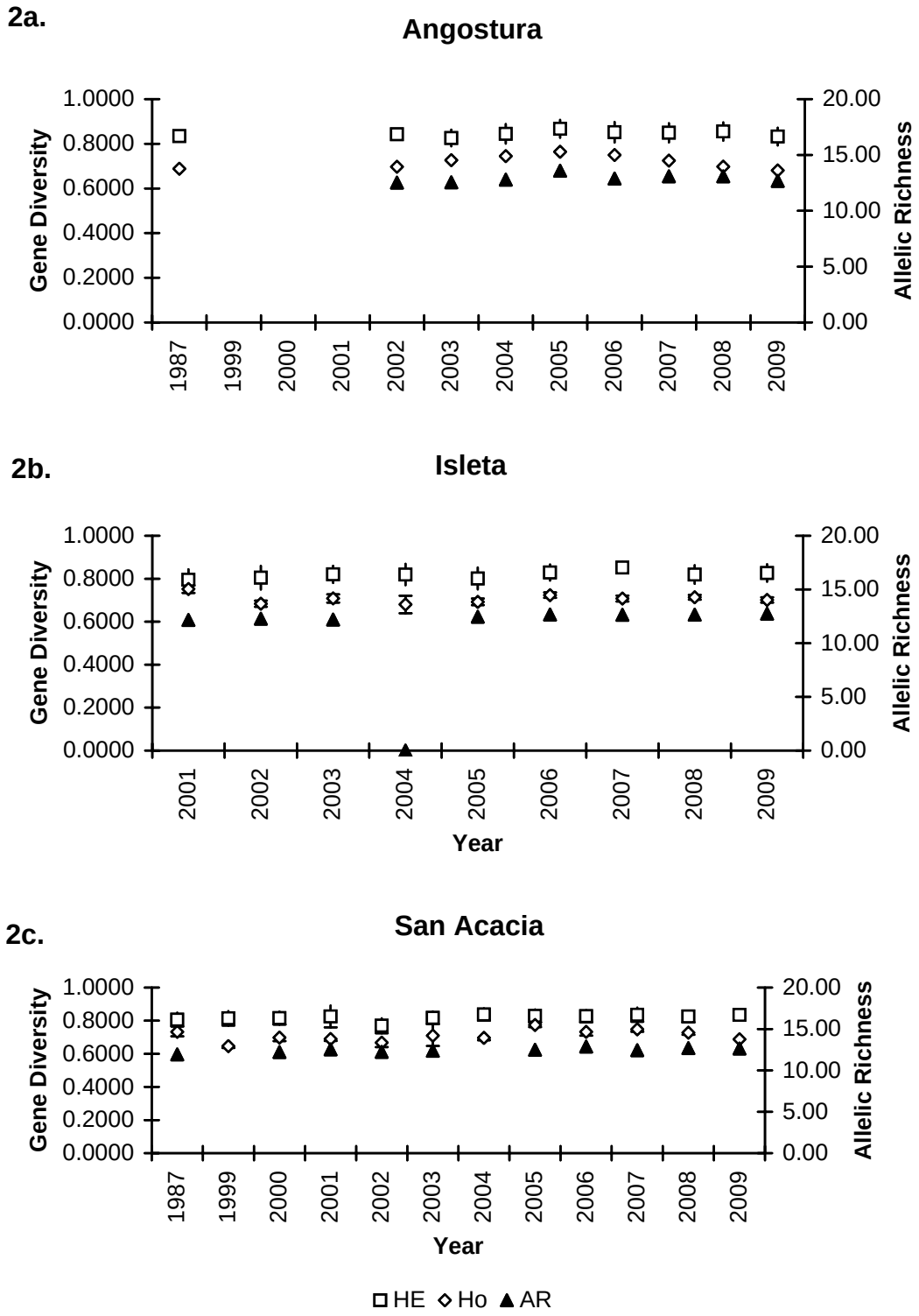


Figure 2. Diversity statistics for wild fish by river reach (a) Angostura, (b) Isleta and (c) San Acacia. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness (A_R) are shown. Standard deviation bars are given for H_E and H_O.

Captive reared fish released to Big Bend National Park, Texas

Measures of genetic diversity (H_E, A_R, 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. The number of MtDNA haplotypes identified in these captive stocks ranged from six to nine. A previously unidentified haploype (W) was identified in a single stock. Haplotype richness was lower than in the wild samples whilst *h* was similar or in some cases slightly higher. Pairwise F_{STs} calculated among the eight lots of captive-spawned fish revealed no significant variation among the four lots of fish released from Dexter National Fish Hatchery (MJO09-001, MJO09-002, MJO09-003, MJO09-004). For the fish released from the Albuquerque Biological Park, MJO08-009 was significantly different from MJO08-006 and MJO08-007. Average F_{ST} was 0.0132 when batches of fish released from Dexter were compared to those released from the Albuquerque Biological Park and 14 of 16 pairwise comparisons were significantly different from zero after Bonferroni correction was applied. Values of Φ_{ST} calculated among all pairs of captive-bred fish released to Big Bend were small and none were significant.

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 14 of 66 total comparisons were significant (Table 4a). Genetic variation among these samples was small but significantly different from zero (F_{CT} = 0.0028 P < 0.0001). A significant portion of genetic variation could be explained by differences among samples within groups (F_{SC} = 0.009, P < 0.0001), and among samples irrespective of their groupings (F_{ST} = 0.0118, P < 0.0001). Pairwise F_{STs} also were calculated for 2009 among sampling localities. Values of F_{ST} were small yet three were significant after Bonferroni correction for multiple tests from a total of 66 pairwise comparisons (Table 4c). Hierarchical analyses of molecular variance was conducted by grouping samples by river reach. Genetic differences among river reaches were not statistically different from zero (F_{CT}=0.00005, P=0.6816)

Mt-DNA- Population Structure

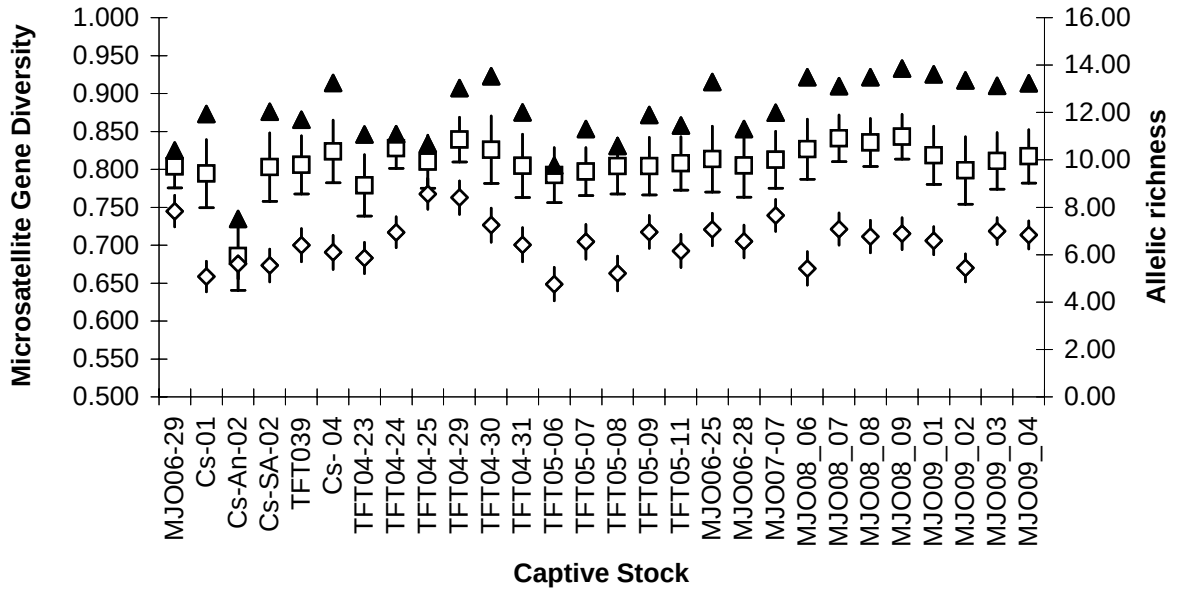
Pairwise Φ-statistics were calculated between all wild samples collected in 1987 and from 1999-2009. After Bonferroni correction was applied, three significant comparisons were identified from a total of 66 comparisons (Table 4b). The significant comparisons involved the 2002 sample. We also conducted two hierarchical analyses of molecular variance in which samples were grouped by year and by river reach. Results indicated that genetic differences among river

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	0.076	0.028	0.064	0.034	0.006	0.004	0.017	-	0.002	0.011	-	0.002	-	-
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	-	-	-	-	-	-	-	-	-	-
TFT04-25	0.434	0.057	0.113	0.057	0.283	0.057	-	-	-	-	-	-	-	-	-	-

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

3a



3b

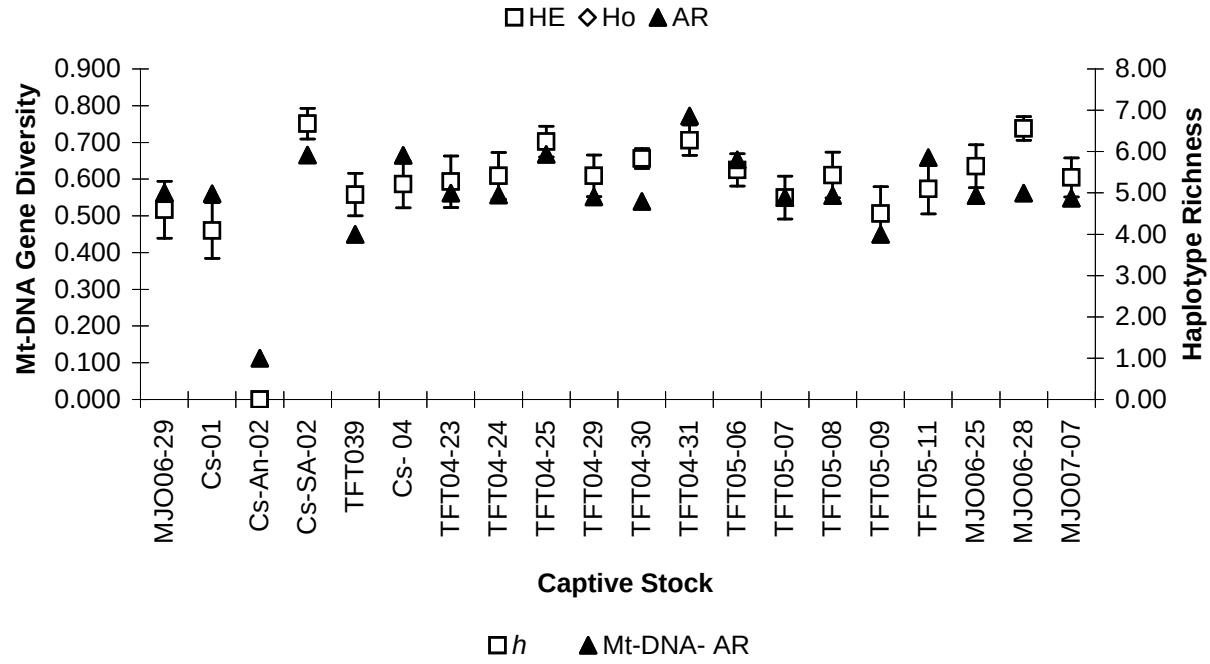
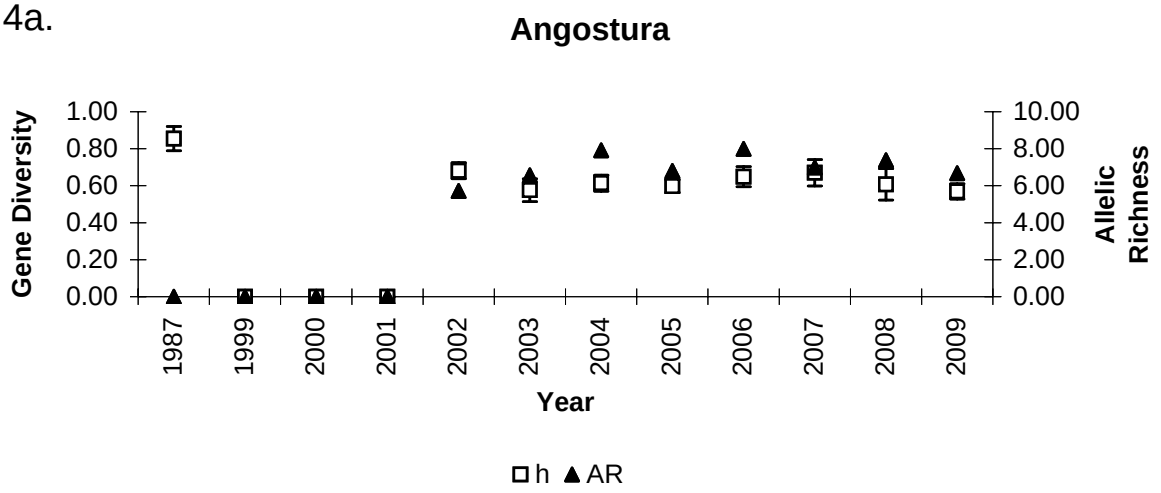
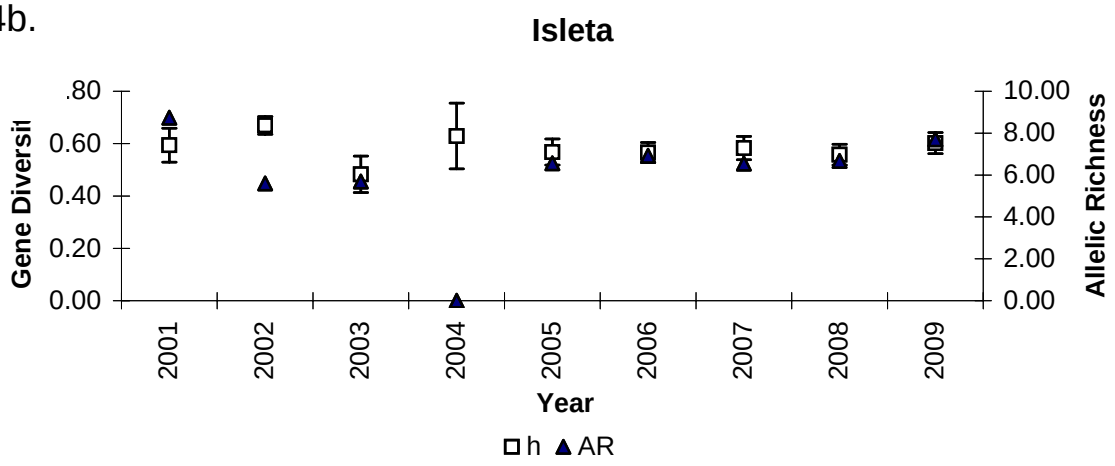


Figure 3. Diversity statistics for captive stocks. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (*h*) and haplotype richness are given (**b**). Standard deviation bars are given for *h*, H_E and H_O.

4a.



4b.



4c.

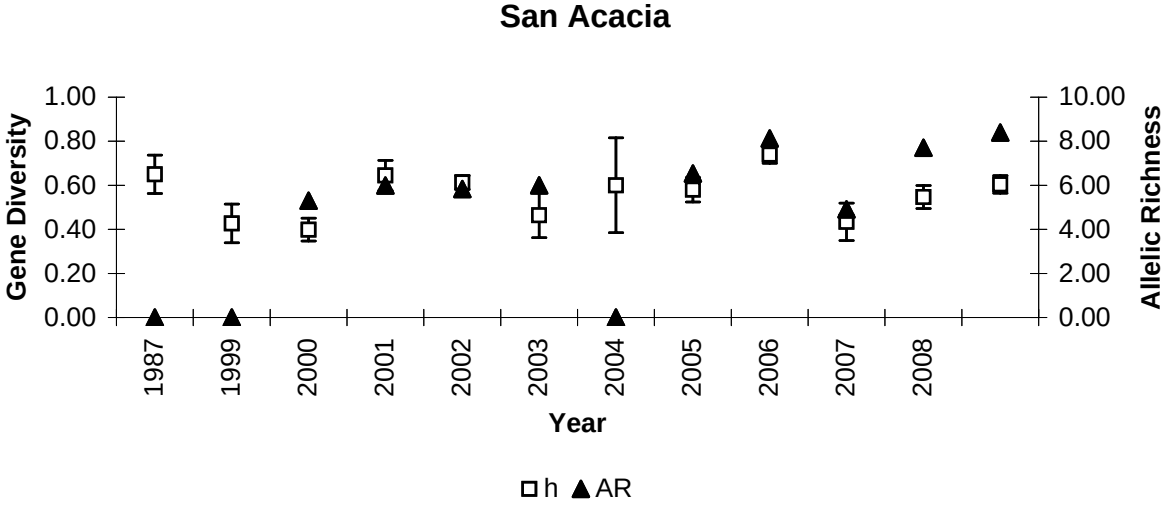


Figure 4. Diversity statistics for wild fish by river reach (a) Angostura, (b) Isleta and (c) San Acacia. For Mt-DNA average gene diversity (*h*) and allelic richness. (A_R) are shown. Standard deviation bars are given for *h* .

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 $F_{ST}s$ calculated among sampling localities for microsatellites and d) mitochondrial DNA. Shading indicates significant values after Bonferroni correction. San Acacia DD- San Acacia Diversion Dam.

a.

	1987	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
1987	*	0.999	0.999	0.999	0.990	0.999	0.999	0.999	0.999	0.999	0.999	0.999
1999	-0.032	*	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
2000	-0.007	-0.027	*	0.200	0.000	0.903	0.826	0.997	0.004	0.017	0.101	0.000
2001	-0.009	-0.027	0.001	*	0.000	0.999	0.999	0.998	0.557	0.056	0.879	0.098
2002	-0.003	-0.027	0.004	0.003	*	0.000	0.001	0.001	0.000	0.000	0.000	0.000
2003	-0.010	-0.036	-0.001	-0.003	0.002	*	0.961	0.999	0.999	0.999	0.999	0.000
2004	-0.013	-0.040	0.000	-0.004	0.002	-0.001	*	0.999	0.995	0.299	0.980	0.000
2005	-0.011	-0.026	-0.001	-0.003	0.001	-0.006	-0.004	*	0.999	0.988	0.999	0.000
2006	-0.011	-0.028	0.002	0.000	0.004	-0.003	-0.001	-0.001	*	0.004	0.003	0.000
2007	-0.012	-0.035	0.002	0.001	0.007	-0.002	0.001	-0.001	0.002	*	0.000	0.000
2008	-0.009	-0.032	0.001	-0.001	0.006	-0.003	-0.001	-0.002	0.001	0.002	*	0.001
2009	-0.014	-0.033	0.002	0.001	0.007	0.002	0.006	0.001	0.006	0.005	0.001	*

b.

	1987	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
1987	*	0.138	0.010	0.010	0.287	0.181	0.045	0.031	0.107	0.020	0.009	0.017
1999	0.020	*	0.370	0.258	0.076	0.679	0.518	0.291	0.364	0.316	0.282	0.221
2000	0.078	-0.002	*	0.253	0.000	0.023	0.080	0.019	0.010	0.097	0.046	0.019
2001	0.059	0.004	0.002	*	0.003	0.047	0.344	0.199	0.047	0.610	0.480	0.311
2002	0.003	0.018	0.049	0.030	*	0.046	0.021	0.003	0.044	0.007	0.000	0.000
2003	0.010	-0.009	0.019	0.016	0.008	*	0.286	0.086	0.224	0.103	0.029	0.026
2004	0.027	-0.004	0.011	0.000	0.011	0.001	*	0.727	0.469	0.881	0.682	0.562
2005	0.031	0.002	0.012	0.002	0.012	0.005	-0.002	*	0.147	0.787	0.359	0.614
2006	0.016	-0.001	0.021	0.009	0.005	0.002	-0.001	0.002	*	0.157	0.089	0.121
2007	0.041	0.001	0.008	-0.003	0.015	0.006	-0.004	-0.002	0.002	*	0.937	0.823
2008	0.049	0.003	0.010	-0.001	0.018	0.009	-0.002	0.000	0.003	-0.003	*	0.924
2009	0.044	0.005	0.013	0.001	0.015	0.009	-0.001	-0.001	0.002	-0.002	-0.002	*

4c.

	Sandia	Dixon Lomitas	AMACFA	Central	Isleta DD	Alejandro	Los Lunas	San Acacia DD	San Antonio	San Marcial
Sandia	*	0.0000	0.9932	0.1797	0.0479	0.0879	0.1260	0.0518	0.7402	0.6572
Dixon_Lomitas	0.0115	*	0.0430	0.0889	0.0762	0.0361	0.0889	0.0000	0.0576	0.0000
AMACFA	-0.0038	0.0082	*	0.2236	0.1035	0.1973	0.6826	0.1514	0.6826	0.3565
Central	0.0038	0.0044	0.0055	*	0.3203	0.6104	0.7666	0.1514	0.8135	0.1221
Isleta DD	0.0047	0.0041	0.0066	0.0023	*	0.5547	0.0693	0.0068	0.2578	0.3857
Alejandro	0.0046	0.0051	0.0049	0.0011	0.0009	*	0.3457	0.0273	0.4893	0.5156
Los Lunas	0.0041	0.0043	0.0011	0.0001	0.0045	0.0022	*	0.1250	0.7861	0.0830
San Acacia DD	0.0054	0.0137	0.0060	0.0036	0.0071	0.0055	0.0039	*	0.0332	0.1973
San Antonio	0.0005	0.0053	0.0017	0.0001	0.0029	0.0017	0.0002	0.0064	*	0.7158
San Marcial	0.0007	0.0101	0.0037	0.0038	0.0017	0.0013	0.0045	0.0031	0.0006	*4

d.

	Sandia	Dixon Lomitas	AMACFA	Central	Isleta DD	Alejandro	Los Lunas	San Acacia DD	San Antonio	San Marcial
Sandia	*	0.0488	0.3809	0.0723	0.0869	0.0293	0.3457	0.0068	0.3135	0.0156
Dixon_Lomitas	0.0343	*	0.5195	0.9815	0.9629	0.5440	0.2207	0.7139	0.6563	0.8350
AMAFCA	0.0004	-0.0108	*	0.7588	0.6807	0.7295	0.9199	0.5381	0.9395	0.4785
Central	0.0241	-0.0176	-0.0185	*	0.9961	0.6709	0.3506	0.6533	0.8682	0.7539
Isleta DD	0.0231	-0.0173	-0.0156	-0.0176	*	0.7305	0.4170	0.5527	0.8018	0.6787
Alejandro	0.0392	-0.0072	-0.0181	-0.0105	-0.0118	*	0.4150	0.5449	0.4600	0.6221
Los Lunas	0.0019	0.0059	-0.0248	0.0004	-0.0023	-0.0013	*	0.1494	0.8018	0.1211
San Acacia DD	0.0601	-0.0120	-0.0101	-0.0103	-0.0077	-0.0066	0.0151	*	0.3604	0.9492
San Antonio	0.0043	-0.0101	-0.0251	-0.0130	-0.0130	-0.0042	-0.0126	0.0003	*	0.3174
San Marcial	0.0591	-0.0149	-0.0064	-0.0119	-0.0111	-0.0095	0.0166	-0.0169	0.0026	*

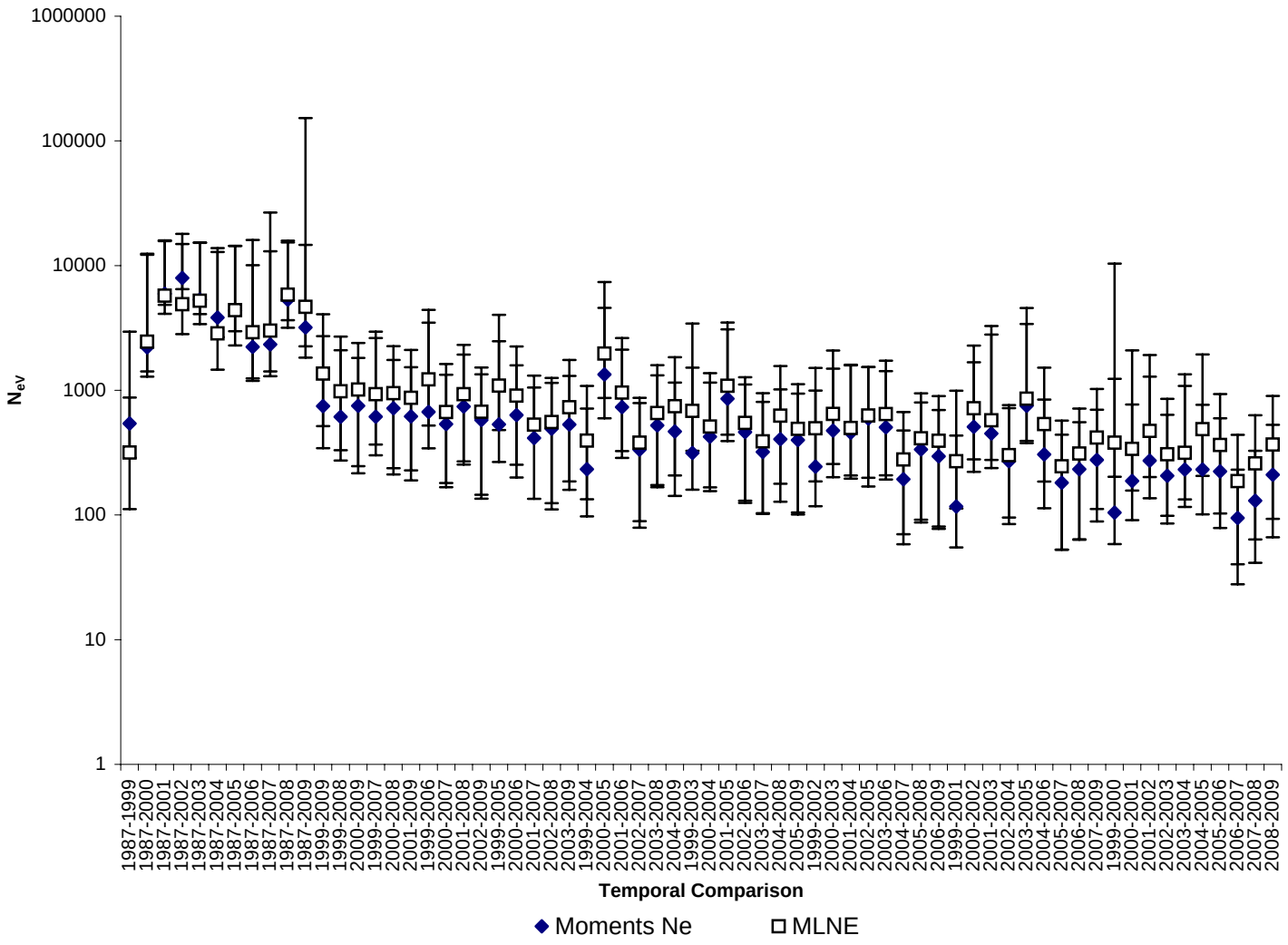


Figure 5. Pairwise effective size estimates calculated from microsatellite DNA data. Moments-based and pseudo-maximum likelihood (MLNE) estimates are given. 95% confidence intervals are shown.

Table 5. Pairwise genetic effective size estimates (MLNE and Moments-based) from mitochondrial DNA data. 95% Confidence intevals are given.

Temporal Comparison	$N_{ef\ (MLNE)}$	-95%CL	+95%CL	$N_{ef(Moments)}$	-95%	+95%
1987-1999	62.51	19.69	425.26	87.1	11.4	Infinity
1987-2000	72.79	29.44	219.56	72.3	11.4	384.5
1987-2001	878.93	148.57	Infinity	359.1	47.6	Infinity
1987-2002	450.82	127.12	Infinity	709.4	45.3	Infinity
1987-2003	502.4	128.7	Infinity	457	52.5	Infinity
1987-2004	2071.26	206.63	Infinity	683.6	71.6	Infinity
1987-2005	Infinity	371.11	Infinity	Infinity	161.8	Infinity
1987-2006	Infinity	413.35	Infinity	Infinity	157.2	Infinity
1987-2007	1172	232.3	Infinity	908.7	90.6	Infinity
1987-2008	1961.5	320.4	Infinity	1542	128.8	Infinity
1987-2009	24038.32	510.1	Infinity	46486.6	198	Infinity
1999-2009	Infinity	335.6	Infinity	309.4	53.6	Infinity
1999-2008	28248.9	272.1	Infinity	302.4	46.1	Infinity
2000-2009	144.4	41.4	422.4	430.8	172.9	2296.6
1999-2007	947.4	190.3	Infinity	243.9	33.8	Infinity
2000-2008	378.31	140.2	2958.7	268.8	41.1	Infinity
2001-2009	Infinity	570.2	Infinity	Infinity	313.6	Infinity
1999-2006	2113.86	166.06	Infinity	162.7	26.7	Infinity
2000-2007	366.4	118.2	Infinity	119.1	27.5	476.6
2001-2008	1710.9	252.7	10000	Infinity	240.1	Infinity
2002-2009	930.8	319.9	37734.2	358.2	94.3	1382.6
1999-2005	Infinity	109.77	Infinity	114	20.5	Infinity
2000-2006	177.63	78.46	585.81	69.1	17.7	200
2001-2007	9954.8	216.6	10000	Infinity	195.7	Infinity
2002-2008	558.9	218.9	2896.3	285.6	76.3	1043.4
2003-2009	1396.32	270.9	Infinity	308.8	69.2	3675.3
1999-2004	Infinity	103.7	Infinity	134.3	19.3	Infinity
2000-2005	163.83	70.27	619.21	65.9	16.6	200.7
2001-2006	465.28	121.03	Infinity	503.8	66.1	Infinity
2002-2007	580.3	171.3	Infinity	229.3	51.4	1539.6
2003-2008	528.3	156.1	Infinity	265.2	58.8	3747.1
2004-2009	796539	315.9	Infinity	Infinity	77.7	Infinity
1999-2003	Infinity	122.5	Infinity	Infinity	27	Infinity
2000-2004	146.75	56.1	1167.36	81.9	15.8	554.3
2001-2005	493.86	101.57	Infinity	508.1	52.4	Infinity
2002-2006	310.05	126.95	1554.27	144.1	37.2	490.9
2003-2007	1487.9	149	Infinity	419.5	54.5	Infinity
2004-2008	819.8	183.5	Infinity	Infinity	119.5	Infinity
2005-2009	49081.8	682.2	Infinity	Infinity	310.9	Infinity
1999-2002	130.08	44.7	Infinity	25.5	4.6	131.5
2000-2003	248.27	64.15	Infinity	61.4	11.8	415.8
2001-2004	Infinity	105.1	Infinity	Infinity	56.7	Infinity
2002-2005	184.53	84.47	641.36	91.6	22.5	298.4
2003-2006	253.03	84.21	Infinity	106.7	22.4	711.9
2004-2007	9975.5	138.8	Infinity	Infinity	139.3	Infinity
2005-2008	1404	181.7	10000	533.2	88.5	Infinity

Table 5 cont.

Temporal Comparison	$N_{ef\ (MLNE)}$	-95%CL	+95%CL	$N_{ef(Moments)}$	-95%	+95%
2006-2009	49696.4	447.4	49696.4	Infinity	185.1	Infinity
1999-2001	Infinity	59.08	Infinity	53.9	7.5	Infinity
2000-2002	48.86	29.68	97.87	11.2	2.5	30.3
2001-2003	96.26	35.22	Infinity	73.6	12.8	Infinity
2002-2004	192.54	78.2	Infinity	59.3	14.3	266
2003-2005	207.29	61.5	Infinity	82.21	17.1	1021.8
2004-2006	306.2	79.63	Infinity	290.5	34.8	Infinity
2005-2007	6653.9	106.7	Infinity	1863.3	54.4	Infinity
2006-2008	927.4	199.2	Infinity	538.7	69.4	Infinity
2007-2009	49717.9	285.6	49717.9	Infinity	138.1	Infinity
1999-2000	Infinity	26.98	Infinity	Infinity	4.4	Infinity
2000-2001	172.3	42.46	Infinity	19	4	125.1
2001-2002	66.89	34.86	232.94	22.1	5	100.5
2002-2003	87.37	43.16	507.12	18.9	4.2	67.7
2003-2004	Infinity	61.59	Infinity	122.8	13.3	Infinity
2004-2005	343.97	58.55	Infinity	533.8	24	Infinity
2005-2006	355.81	87.91	Infinity	133.6	22.5	Infinity
2006-2007	953.8	89.5	Infinity	128.4	18.5	Infinity
2007-2008	49958.7	134.1	49958.7	Infinity	80.8	Infinity
2008-2009	49747.4	234.2	49747.4	Infinity	169.7	Infinity

reaches (Angostura, Isleta and San Acacia) (Φ_{CT} = -0.0015, P = 0.9226) were not significantly different from zero. When samples were grouped by years a small but significant (Φ_{CT} = 0.0076, P=0.0007) portion of variation could be explained by year. We also calculated pairwise Φ_{ST} values among 2009 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 2009 (Figure 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 2008-2009 comparison, N_{eV} was 210.1 (95 % CIs 143.8 – 318) and MLNE estimate was 367.3 (95 % CIs 274.3 – 533.6) for the most recent temporal comparison (2008-2009). The harmonic mean across all pairwise estimates (1999-2009) was 495.8 (pseudo-maximum-likelihood) and 317.5 (N_{eV}).

Variance female effective size (calculated from Mt-DNA haplotype frequencies) continued to be high with a moments based estimate of infinity for 2007-2008 and 2008-2009 comparisons from an N_{ef} of 128.4 and 953.8 (MLNE) for 2006-2007 comparison (Table 5). The harmonic mean for samples collected from 1999 to 2009 was N_{ef} = 288.654 (pseudo-maximum likelihood) and N_{ef} = 77.319 (moments-based) (samples where N_{ef} was infinity, were excluded).

Discussion

Genetic status of the species in 2009

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 2009 average gene diversity increased above that recorded in 2008 and microsatellite allelic richness remained stable from 2006-2009 in the wild Rio Grande silvery minnow population. The level of gene diversity at the mitochondrial ND4 gene was similar to that recorded in 2008 whilst allelic richness was higher in 2009 compared to values recorded between 2005 and 2008. More mitochondrial haplotypes were identified in 2009 than in prior years. 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 2008-2009 calculated using the temporal method, increased over values recorded for the previous period (2007-2008). Despite the increase in genetic effective size it is still a fraction of the estimated census size of the population made in October 2008 (Dudley et al. 2009). 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 mitchondrial DNA haplotype frequency data showed a dramatic increase in female effective size for the 2007-2008 and 2008-2009 comparisons over previous years. There are several possible explanations for the discrepancy between mitochondrial and nuclear effective size estimates 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 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 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 2009 we characterized genetic variation in eight 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 and comprised age 0 and age 1 fish. The fish bred at Dexter were a mixed lot (CsDx07 and CsDX08). CsDx07 were produced using broodstock (240 pairs) collected in 2002 as eggs. CsDx08 were produced using broodstock (172 pairs) collected from 2002-2005 as eggs and juveniles. 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 2009 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 such 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 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) no statistically significant spatial genetic structure was identified 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 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). In 2008, fish were only stocked in the spring and no stocking has occurred so far in 2009. Interestingly, heterozygosity and microsatellite allelic richness have been generally higher from 2005-2009 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.

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