Genetic Monitoring of the Rio Grande Silvery Minnow: Genetic Status in 2006

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Executive Summary

We report on the genetic status of wild and captive stocks of Rio Grande silvery minnow in calendar year 2006. In 2006, roughly 400 adult fishes were non-destructively sampled from the Rio Grande and assessed for genetic variability at nine microsatellite DNA loci and the ND4 region of the maternally-inherited mitochondrial DNA. Fishes sampled in 2006 represent the eighth consecutive generation for which genetic data have been obtained on wild stocks. In addition, 21 captive stocks of Rio Grande silvery minnow have been sampled since the inception of the captive breeding and augmentation program. Over this time span there has been considerable environmental variation in the middle Rio Grande and corresponding fluctuations in abundance of Rio Grande silvery minnow. For example, mean discharge varied by several orders of magnitude over the last eight years from extreme lows from 1999 to 2003 to high snowmelt discharge in 2005. Discharge appears to be positively related to abundance of Rio Grande silvery minnow in the wild, and monitoring data shows striking increases in abundance in 2005 coincident with high snowmelt flows. Apparent increases in abundance are almost certainly related to population supplementation from stocks reared and/or spawned in captivity and released into the wild, a practice known as supportive breeding. The demographic effect of supportive breeding is straightforward in that we expect census numbers to increase as captively-bred fishes are released. In contrast, the genetic effects of supportive breeding are complicated and depend on a number of factors, including population dynamics and genetic characteristics of captive and wild stocks.

Our study focused on genetic response of wild Rio Grande silvery minnow stock to supportive breeding and supplementation, and ostensibly to increased wild abundances in 2005. For microsatellites, allelic richness and expected heterozygosity declined slightly as did haplotype richness and gene diversity in the protein-encoding ND4 region of the mitochondrial DNA. However, calendar year 2006 marks the forth consecutive silvery minnow generation where there is a general trend toward increased allelic richness and heterozygosity after declines in 1999, 2000, 2001 and 2002. Our conclusion is that increased diversity resulted from supportive breeding as alleles were identified in 2006 that had been reduced to low frequencies or had been lost in the wild in previous generations, but had been maintained in captive stocks and subsequently reintroduced to the wild. An increase in allelic richness implies an increase in the inbreeding genetic effective size (N_{el}) in 2005 over previous years, because of increased diversity of parents that included both wild and captive individuals. In addition, our results imply that captive stocks that originated from wild-caught eggs, adults and captively-bred individuals have been an important reservoir for genetic diversity for the species during a period of reduced diversity (e.g. from 1999-2002) and periods of depressed population size in the wild. However, variance effective size (N_{eV}) in wild populations remains low (N_{eV} = 122, lower 95% CI = 90, upper 95% CI = 168) suggesting 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 wild parents. Consequently, gains made in genetic diversity to the wild population observed in 2004-2006 will be lost in a few generations if supportive breeding and supplementation ceases in the absence of correcting the underlying causes of population decline.

Theory shows that multiple generations of supportive breeding can have positive effects on genetic diversity, but thus far only very simple models have been developed to predict the maintenance of diversity and the magnitude of effective size. Future studies in our laboratory will refine these models for Rio Grande silvery minnow, based on knowledge of diversity of captive and wild stocks obtained from our extensive database. Refinement of models can aid in management recommendations for maintenance of captive broodstock. However, genetic data from 2006 unequivocally demonstrates that strategies implemented in propagation and supplementation programs, namely, maintaining multiple captive brood stocks and periodic refreshment of brood stock with wild-caught individuals (eggs, larvae, or adults) has served as a genetically diverse source for supplementation of wild stocks in the last three years.

Introduction

Desert rivers of the American Southwest are highly impacted by anthropogenic factors including physical modification resulting from construction of diversion structures and reservoirs which fragment the river, habitat destruction including channelization and alteration of flow regimes including extended periods of river dewatering. Fish species are not impacted equally by these factors with some adversely affected whilst others thrive. A guild of fishes referred to as pelagic broadcast spawners tend to fall into the former category. For example, in the Rio Grande of New Mexico, river drying and dam construction are thought to be responsible for the final demise of several species within this reproductive guild including the phantom shiner (*Notropis orca*) and the Rio Grande bluntnose shiner (*Notropis simus simus*) (Bestgen and Platania 1990). Several other pelagic species have been extirpated from the New Mexico portion of the Rio Grande including the Rio Grande shiner (*Notropis jemezanus*) and speckled chub (*Macrhybopsis aestevalis*). The middle Rio Grande, New Mexico is fragmented by three water diversion structures and two reservoirs which divide the river into four discrete reaches: i) Cochiti (35.9 km), ii) Angostura (65.2 km), iii) Isleta (85.5 km) and iv) San Acacia (90.4 km). Pelagic spawners suffer from the construction of impassable dams as they spawn in response to increases in flow that occur in spring (Platania and Altenbach 1998) and their neutrally buoyant, pelagic eggs then drift with river currents resulting in displacement of propagules downstream. Upstream movement of fish is precluded by diversion structures. Without intervention, populations in upstream reaches gradually decline and may disappear entirely if there is little retention of eggs and larval fish in upstream areas (Winston et al. 1991; Luttrell et al. 1999). In the Rio Grande, populations of Rio Grande silvery minnow in downstream reaches are threatened annually because of prolonged and extensive river drying in these areas. Drying tends to occur immediately after the Rio Grande silvery minnow spawns and irrigation commences. These dynamics have important demographic and genetic consequences.

Routine population monitoring for the Rio Grande silvery minnow commenced in the mid 1990's and revealed a dramatic decline in abundance when compared to data collected in the 1980's (Platania 1991). As a result, the species was listed as endangered under the Endangered Species Act in 1994. Genetic monitoring began in 1999 and has continued annually since this time. Following Swartz et al. (2006) we define genetic monitoring as quantifying temporal changes in genetic parameters using molecular methods. Genetic monitoring has the potential to reveal information that may not be obtainable with traditional monitoring techniques or with genetic studies that lack a temporal dimension. Temporal genetic studies track changes in genetic diversity parameters such as allelic diversity and heterozygosity and allow the effective population size to be tracked (e.g. Hauser et al. 2002; Hoarau et al. 2005; Hutchinson et al. 2003; Saillant & Gold 2006). Such data allows the impact of population decline and of management activities to be assessed from a genetic perspective.

As a population declines genetic diversity is lost as a result of increased rates of genetic drift between generations. Rare alleles are more likely to be lost than alleles that occur at higher frequencies in the population. The smaller a population becomes and the longer it remains at this size, the greater the loss of genetic variation. Preservation of diversity is critical if a species is to be able to persist over the long-term as genetic variation forms the basis of a species' ability to adapt to changing environmental conditions (Frankel & Soulé 1981). For example, diversity in genes of the major histocompatability complex (MHC) is central to an individuals' ability to respond to viral, bacterial and parasitic infection. Absence of diversity at these loci may place a population at increased risk of extinction if novel pathogens emerges. Measures of genetic diversity include average number of alleles per locus (A) (alternatively allelic richness [A_R] which corrects for unequal sample size), temporal change in allele frequencies (F_k, Waples 1989) (Leberg 1992) and heterozygosity. Although population bottlenecks affect all components of genetic variation, allelic diversity is impacted more rapidly than heterozygosity (Nei et al.1975). Heterozygosity will be reduced if the population persists at low levels for several generations. Species characterized by short generation lengths, such as many small-bodied minnows, are expected to experience greater

losses per year for a given population size (Amos & Balmford 2001). It is generally thought however, that such species exist at higher population densities with large effective population sizes (Amos & Balmford 2001).

The rate at which genetic diversity is lost is directly proportional to the effective population size. The effective population size is defined as the number of adult individuals that successfully contribute genes to subsequent generations and in most cases N_e is a fraction of the census size (Frankham 1995). In some instances N_e may be orders of magnitude smaller than the census size (Bagley et al. 1999; Hauser et al. 2002; Li and Hedgecock 1998; Hoarau et al. 2005, Turner et al. 2002; Saillant & Gold 2006) and is the case for Rio Grande silvery minnow (Alò and Turner 2005). For example, in highly fecund species the population may be large yet still characterized by a small effective size in cases where relatively few individuals contributed to the next generation. Hence, genetic monitoring of the population can reveal whether a species is likely to be at risk of extinction from genetic factors such as in populations that are experiencing rapid rates of genetic diversity (if N_e is small) (e.g. Proctor et al. 2005; Miller & Waites 2003; Nussey et al. 2005;Shaklee et al. 1999).

Theoretical studies have indicated that under certain circumstances supportive breeding may exacerbate reduction of the effective population size for the population as a whole (Ryman & Laikre 1991). This may occur if a large number of fish produced from a few breeders are successfully introduced into the wild (Ryman & Laikre 1991; Waples 1991; Waples & Do 1994). This is referred to as the Ryman-Laikre effect. Alternatively, captive breeding and stocking programs may help to maintain diversity in the population with the captive population acting as a reservoir for diversity. Captive breeding and rearing has been a central recovery action for the Rio Grande silvery minnow. These efforts were instigated to provide refugial populations to prevent species extinction in the event of a catastrophic loss of the wild population, and to provide large numbers of fish that could be used in supportive breeding activities designed to increase the number of fish in the wild. Theoretical data on the predicted genetic consequences of hatchery propagation and supplementation are plentiful (e.g. Wang & Ryman 2001; Ryman & Utter 1987; Laikre & Ryman 1996; Ryman & Laikre 1991; Waples & Do 1994) yet there are few empirical genetic studies to examine supportive breeding.

This study reports genetic monitoring data collected across eight consecutive years for the Rio Grande silvery minnow, and an additional sample that was taken in 1987. We also sampled 21 different captive stocks that served as the source of fish for supplementation of the wild population. This study represents one of the longest running genetic monitoring data sets from a non-salmonid freshwater fish and provides invaluable insights into the genetic effects of population decline and of supportive breeding.

Methods

Sampling-
Rio Grande Population

The wild population was sampled annually from 1999 to 2006 (between December and April). 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) (Table 1, referred to as 1987 sample) were genotyped. Throughout this study 'wild' means an unmarked fish sampled from the Rio Grande. These fish may have parents that were bred or reared in captivity. 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 and 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 21 different captive stocks (four stocks from captively-reared wild caught eggs and 16 stocks from captive spawning) collected between 2000 and 2005.

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). Individuals were also screened for variation at three additional loci (Lco4, Lco5 and Ca1). Analysis of family groups demonstrated that alleles at these loci either did not segregate according to Hardy-Weinberg expectations or could not be scored reliably so these were not included in further analyses. 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 94°C for 2 mins followed by 30 cycles of 94 °C for 20s, 50°C for 20 s, 72°C for 30s. For *Ppro 118*/*Ppro126* cycling conditions were one denaturation cycle of 94°C for 2 mins followed by 30 cycles of 94 °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 ROX350 size standard (12.5%) and loading buffer (25%) and denatured at 93 °C for 2 minutes. Samples were run on an ABI 377 automated DNA sequencer at 50°C for 2.5 hours. *Ppro 118*/*Ppro126* 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 was 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 as described in Alò and Turner (2005).

Statistical Analysis

Microsatellite data was 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 the 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 populations. 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. Percent sequence divergence was estimated using Kimura-two parameter method as implemented in PAUP Version 4.0b10 (Swofford 2001). The computer program TCS (Clement et al. 2000) was used to construct a statistical parsimony network among mitochondrial DNA haplotypes using the method of Templeton *et al*. (1992).

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, captively spawned, and captively 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) implemented in 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 method and MLNE. 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 fish movement is precluded by dams and these species are rarely taken upstream of the study area (J. Brooks, USFWS, pers. comm).

Temporal-method estimates of N_e and N_{ef} were calculated from *F'* values obtained from all possible pairs cohorts sampled from 1987 to 2006, 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.

Results

Microsatellites- Genetic Diversity

Genotype data from nine microsatellite loci and 3146 wild and captive Rio Grande silvery minnow is presented here. All of the microsatellite loci used in this study were highly polymorphic with 10 (*Ppro126*) to 57 (*Ppro118*) alleles per locus (Table 1). Allelic richness (A_R) ranged from 13.56 to 15.13 among the wild samples (A_R was not calculated for 1987 and 1999 samples due to small number of samples) (Table 2, Fig. 1a). Among the captive stocks reared from wild-caught eggs A_R ranged from 12.05 to 14.90 and among the captive spawned stocks A_R ranged from 8.37 to 14.54. Gene diversity (H_e) was between 0.808 (1987) and 0.864 (2005). In general, gene diversity and allelic richness (and effective number of alleles) increased slightly between 1999 and 2006. When comparing the wild population, stock reared from wild-caught eggs and captively spawned stocks average allelic diversity is highest in the wild population while average gene diversity is highest in the wild caught eggs (Fig. 1a, 2a). In the Angostura reach A_R increased between 2002 and 2005 but declined in 2006 (Figure 3). In the Isleta reach A_R increased from 2001 to 2002 but no obvious trends for H_O or H_E were observed. In the San Acacia reach A_R increased from 2000 to 2006 and H_E increased slightly over the sampling period. Neither A_R, H_E, H_O or F_{IS} differed significantly between samples collected in different river reaches.

Among wild samples there were 42 departures (after Bonferroni correction) (Rice 1989) from Hardy-Weinberg expectations from 81 comparisons. *Ppro118* was the only loci that conformed to HWE expectations in all wild populations. Among captive stocks (captively spawned and captively reared wild caught eggs) there were 82 significant departures from HWE (after Bonferroni correction) from 189 comparisons. Microchecker indicated that null alleles were the most likely cause for deviation from HWE. After Bonferroni correction for multiple univariate tests, there were seven cases of significant linkage disequilibrium. Linkage disequilibrium was observed between *Lco6* and *Lco7*/*Ca8*, between *Lco1* and *Ppro118*/*Ppro126* and between *Ppro126* and L*co8*/ *Ca8*/ *Ppro118*. Linkage disequilibrium among otherwise unlinked genetic loci is expected when N_e is relatively small (Waples 2006).

MtDNA- Genetic Diversity

Haplotype data was obtained for 3010 individuals identifying 14 mt-DNA ND4 haplotypes from 295 base pairs of sequence. Haplotypes differed by one to seven nucleotide changes (0.341% to 2.431% sequence divergence). Eight haplotypes were separated from haplotype A by single nucleotide changes. Haplotypes N and E were the most divergent with seven nucleotide changes between them. Haplotype A was the most common and was identified as the most likely ancestral haplotype by statistical parsimony analysis (Fig. 4). The frequency of haplotype A ranged from 46% (1987) to 77% (2000) between years (Table 3). Mt-DNA allelic richness ranged from 4.956 (1999) to 9.449 (2004) among wild populations, from 1.000 to 6.865 in captively spawned stocks and from 2.949 to 7.848 in captively-reared wild caught eggs (Table 2, Fig. 1b, Fig. 2b). Gene diversity (h) declined almost 16% from 1987 to 2006.

Microsatellites- Population Divergence

Pairwise F_{STs} were calculated between all wild samples. Values were small yet eighteen were significant (after Bonferroni correction) from a total of 45 comparisons among temporal samples (Table 4a). The 1987, 2000 and 2001 samples were significantly different ($p<0.00001$) from collections made between 2002 and 2006. For hierarchical analysis of molecular variance, data was grouped by river reach and by year. Results of these analyses indicated that genetic differences among river reaches (F_{CT}=0.0016, P=0.1554) were not significantly different from zero in any year tested.

Pairwise values of F_{ST} calculated among stocks reared from wild caught eggs and wild samples. These ranged from 0.0002 to 0.0345, while pairwise values of F_{ST} were between –0.0009 and 0.0966 when calculated among captively spawned stocks and wild samples. For AMOVA

analyses three groups were defined i) wild ii) captively reared wild-caught eggs, and iii) captively spawned stocks. AMOVA indicated that the majority of variation could be explained by difference among samples within groups (F_{SC}=0.01362, P<0.00001).

MtDNA- Population Divergence

A single pairwise value of Φ_{ST} was significant after Bonferroni correction (Table 4b). This was between samples collected in 2000 and 2003. For AMOVA populations were grouped by years and river reach. AMOVA indicated that there was significant divergence between years (Φ_{CT}= 0.0107, P<0.0009) but not between river reaches (Φ_{CT}= -0.0017, P<0.8719). The majority of variation was explained by differences among samples irrespective of their grouping (Φ_{ST}=0.0063, P<0.0303).

Effective Population Size

Variance effective population size (N_{eV}) estimated for wild stocks from temporal changes in allele frequency at nine microsatellite DNA loci was similar to estimates made in previous years beginning in 1999 (Fig 5). Six of eight pairwise comparisons of a sample collected in 1987 with samples collected in 1999 through 2006 indicated a value of N_e > 1000. Pairwise comparisons between samples collected between 1999 and 2006 were uniformly lower, indicating a downward trend in N_e in wild stocks over the last 19 generations. Effective size declined to its lowest values in 1999 and 2000 (N_e = 82) and in 2003-2004 (N_e = 101.4) and leveled off at values ranging roughly between N_e = 100 to 200 thereafter (Fig 5 – based on temporal method estimation) with upper bound 95% confidence intervals generally less than 1000. The MLNE estimation method (Wang 2001) provided larger estimates of N_e than the temporal method in nearly all cases, but both estimation methods indicated consistent trends of decreasing effective size of over the time period from 1987 to 2006. Moreover, harmonic mean N_e = 183 (temporal method) and harmonic mean N_e = 327 (MLNE) estimated across all pairwise comparisons between 2002 and 2006 were both less than a theoretical benchmark N_e = 500. This benchmark value is the effective size required to preserve 95% of selectively neutral genetic variation over evolutionary time scales (Frankham 1995).

Trends in variance female effective size (N_{ef}) based on mtDNA sequence variation mirrored those described for microsatellites above, but there was considerably more variation in estimates across year classes, and 95% CIs were typically broader (Fig. 6). In general, N_{ef} was estimated with lower precision (and possibly less accuracy owing to the presence of many low frequency haplotypes – see Turner et al. 2001) than N_e because the estimate is based on a single locus with fewer independent alleles than microsatellites (see Waples 1989 for discussion of statistical power to estimate N_e). Female effective size was lowest in the period 1999 to 2002, with the lowest values observed in the 2000 – 2002 comparison (N_{ef} = 21 based on temporal-method estimation), and higher between 2003 and 2006 (harmonic mean N_{ef} = 303 [temporal method], harmonic mean N_{ef} = 282 [MLNE]). There were no consistent differences in estimates of N_{ef} derived from the temporal method and MLNE.

Discussion

Genetic diversity of captive stocks

As reported previously eggs collected from natural spawning events display levels of genetic diversity (as measured by allelic richness) that mirror the diversity in the source population. The only exception was eggs collected in the northern Angostura reach which have less diversity than the source population. All other egg collections have been made in the southern end of the range of Rio Grande silvery minnow and reflect the diversity of the whole population. This is because a portion of eggs from spawning adults throughout the range are moved downstream with river currents and as such are represented in egg collections. Stocks from wild caught eggs are also more likely than captively spawned stocks to possess alleles that are found at low frequencies in the wild. The reason for this is simply that greater numbers of eggs can be collected from natural spawning events

which should be the progeny of a larger number of adults provided that there is a strong spawning effort by wild fish. However, although individually each captively spawned stock contains fewer alleles at both microsatellite loci and at a mitochondrial DNA locus, collectively for a given year, levels of diversity as equivalent to that seen in wild-caught eggs.

Genetic status of Rio Grande silvery minnow in 2006

In 2006, the Rio Grande silvery minnow appears to have similar, albeit slightly lower, levels of genetic diversity to those observed in 2005. For microsatellites, allelic richness and expected heterozygosity declined slightly as did haplotype richness and gene diversity in the protein-encoding ND4 region of the mitochondrial DNA. However, calendar year 2006 marks the forth consecutive silvery minnow generation where there is a general trend toward increased allelic richness and heterozygosity after declines in 1999, 2000, 2001 and 2002. We attribute increases in measures of genetic diversity to recovery of low-frequency alleles in the wild that were contributed by supportive breeding and supplementation that began in earnest in 2002.

Captive stocks of Rio Grande silvery minnow originated with eggs and wild adults collected in May 2000. These fish were placed in propagation facilities to act as broodstock and to serve as refugial populations in response to extremely dry conditions and very low abundances of silvery minnow in the wild. Eggs were also collected from natural spawning events in the Rio Grande in 2001, 2002 and 2003. Once raised to adult size, these fish were used to augment the wild population and a portion of each of these stocks was retained to serve as brood fish for future captive stocks. In 2004 and 2005 eggs were not collected from natural spawning events due to high flow conditions so the majority of fish for augmentation efforts conducted between fall 2004 and 2006 were from captive spawning efforts. Previously and here, we have shown that fishes captured as eggs retain much of the diversity present in wild stocks (Osborne et al. 2006), and so continually refreshing the captive broodstock with wild-caught individuals over multiple generations is an important component for maintaining a genetically variable captive broodstock and for prevention of domestication selection. It is also important to retain some fishes from each captive broodstock in a given year to add to the captive broodstock for subsequent years. The latter strategy allows a 'genetic storage' effect (i.e., because of longer life span in the hatchery, brood fishes can contribute genetically to multiple generations) that enhances total diversity of fishes repatriated to the river. Genetic data from wild fishes in 2006 suggest that much genetic diversity apparently lost from the wild in 2000, 2001, and 2002 was protected through collection of eggs from the wild spawning events and maintenance of these fish in captivity. This diversity has subsequently been returned to the river through supplementation. Success at retaining diversity in the hatchery is directly attributable to diligent efforts of facility managers in: (1) minimizing variance in reproductive success among individuals, (2) maximizing the number of breeders in captivity, (3) providing conditions for prolonged life span and multi-year genetic contributions of brood fish, and (4) careful efforts to incorporate newly-captured wild fishes into captive broodstocks. A conceptual representation of multi-year contributions of captive to wild stocks (and vice versa) is presented in Fig. 7. This representation emphasizes strategies implemented by the propagation team. We propose that upward trends in genetic diversity of wild stocks directly results from implementation of these practices. We strongly recommend that these procedures continue as part of the propagation management plan that is under development by the US Fish and Wildlife Service, Dexter National Fish Hatchery and Technology Center (M. Ulibarri and C. Keeler-Foster, pers. comm.). It will be important to maintain large, genetically-diverse captive broodstocks, preferably at several propagation facilities, to provide sufficient progeny to repopulate the Rio Grande in the event of catastrophic loss of Rio Grande silvery minnow in the wild. A value-added component to this strategy is that excess fishes can be used to stock Big Bend. Success of Big Bend stocking is likely to be greatly enhanced by introducing a genetically diverse founding population. (e.g., success of invasion of plains minnow in the Pecos was probably enhanced by diversity of founders – Moyer et al. 2005).

There has been a recent spate of theoretical papers on genetic effects of multiple generations of supportive breeding on endangered focal species (Duchesne & Bernatchez 2002, Wang & Ryman 2001). Results of models presented in these papers indicate that multiple generations of supportive breeding can enhance genetic diversity of wild stocks under conditions that have been implemented by the Rio Grande silvery minnow propagation team. The models, as presented in theoretical papers, evaluate the trajectory of genetic diversity and genetic effective population size under a number of scenarios and characteristics of wild and captive stocks. Unfortunately, none of the scenarios presented adequately reflect conditions for Rio Grande silvery minnow. For example, our research indicates that variance in reproductive success in the wild greatly exceeds variance in reproductive success in the hatchery. High variance of reproductive success in the wild results from a negative interaction of pelagic early life history, downstream transport into unsuitable rearing habitat by river flows, and fragmentation of the middle Rio Grande by dams and diversion structures that prevent upstream migration (Alò and Turner 2005, Osborne et al. 2005, Turner et al. 2006). Future research in our laboratory will evaluate and modify multi-generational models for scenarios that are more applicable to silvery minnow. One specific area of research will be to evaluate models where variance in reproductive success in the wild greatly exceeds variance in reproductive success in captivity.

Effective size of wild stocks in 2006

Pairwise comparisons of allele frequency changes at microsatellites and mtDNA indicate that variance genetic effective size of wild Rio Grande silvery minnow in 2006 remains less than a theoretical benchmark value of N_e = 500, and hovers around 100 to 300. These values of N_e are higher than observed in 1999 to 2002, and increases may have resulted from multi-generational supportive breeding and increased survivorship (and presumably lowered variance in reproductive success) in the wild in 2005. However, there is substantial evidence that low effective size in the wild results from a strong interaction of pelagic early life history and river fragmentation (Alò & Turner 2005, Osborne et al. 2005, Osborne et al. 2006, Turner et al. 2006). Genetic and ecological data obtained for drifting eggs (Dudley 2004; Osborne et al. 2005) and breeding adults (Alò & Turner 2005) are consistent with the idea that reproductive output from most wild breeding pairs is lost from mortality or emigration as eggs and larvae are transported downstream through dams, resulting in high variance in reproductive success and low N_e. Even if larvae survive entrainment, mortality from desiccation occurs because the reaches downstream of both Isleta and San Acacia diversion dams are subject to substantial drying most summers. Drifting eggs maintain genetic "cohesion" as they drift downstream (Osborne et al. 2005), which results in differential (i.e., family-correlated) mortality and enhances variance in reproductive success (Waples 2002). The probability of egg retention in the natal river reach is likely related to the distance to the nearest downstream dam and the magnitude of river flows where spawning occurred (Dudley 2004). A recently published comparative genetic study of Rio Grande silvery minnow, the congeneric plains minnow *(Hybognathus placitus*), and the co-occurring flathead chub (*Platygobio gracilis*) confirms the interaction of life history and fragmentation as a primary cause for low effective size of silvery minnow in the wild (Turner et al. 2006 – see attached reprint). Observed N_e in the 2005-2006 pairwise comparison indicates that high variance in reproductive success continues to depress effective sizes in the wild, despite increased genetic variability overall in wild stocks and an order of magnitude increase in autumnal catch rates for Rio Grande silvery minnow in the wild in 2004 (Dudley et al. 2005a) and for 2005 increases to densities not seen since the species was listed in 1994 (Dudley et al. 2005b).

Genetic results suggest two important factors to consider in future management of Rio Grande silvery minnow. Over the short term, our results imply that multigenerational supportive breeding will be required to maintain a reservoir genetic diversity that counteracts losses of diversity in the wild. Any gains in genetic diversity by wild stocks from supportive breeding are predicted to be quickly lost from the wild population due to high variance in reproductive success in a classic source (i.e., hatchery) and sink (i.e., wild population) dynamic. Secondly, because the ostensible goal for recovery of the Rio Grande silvery minnow is to create conditions that support a self-

sustaining wild population, it will be necessary to reconnect fragmented river reaches and provide sufficient resources (e.g., water & habitat) for successful recruitment of progeny from a greater number of spawning pairs in the wild.

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Table 1. Summary statistics for each wild and captive stock for each microsatellite locus. Sample size (*N*), number of alleles (N_a), allelic richness (A_R) expected heterozygosity (H_E), observed heterozygosity (H_O), and average weighted inbreeding co-efficient (F_{IS}) are given for each locus.

Locus		M1	M3	M6	M7	M8	Ca6	Ca8	Ppro118	Ppro126
1987	**N**	41	42	39	41	41	43	40	33	37
	N_a	28	7	11	10	12	7	23	26	7
	A_R	-	-	-	-	-	-	-	-	-
	H_o	0.829	0.714	0.590	0.732	0.585	0.628	0.625	0.818	0.838
	H_E	0.946	0.721	0.652	0.822	0.870	0.586	0.943	0.942	0.789
	F_{IS}	0.136	0.021	0.108	0.122	0.338	-0.059	0.348	0.147	-0.049
1999	**N**	39	44	42	41	40	33	17	41	42
	N_a	25	8	10	7	11	8	9	27	7
	A_R	-	-	-	-	-	-	-	-	-
	H_o	0.846	0.795	0.595	0.659	0.500	0.515	0.235	0.756	0.714
	H_E	0.946	0.778	0.688	0.783	0.855	0.732	0.851	0.947	0.795
	F_{IS}	0.118	-0.01	0.147	0.171	0.426	0.311	0.738	0.213	0.113
2000	**N**	182	194	193	193	178	187	175	178	175
	N_a	36	12	15	13	17	8	29	48	8
	A_R	24.779	7.39	9.66	9.568	11.61	6.802	19.528	25.711	6.945
	H_o	0.731	0.768	0.627	0.741	0.601	0.684	0.714	0.781	0.811
	H_E	0.957	0.750	0.663	0.827	0.880	0.652	0.936	0.953	0.783
	F_{IS}	0.239	-0.021	0.058	0.107	0.319	-0.047	0.24	0.183	-0.033
2001	**N**	126	126	127	127	125	121	118	117	119
	N_a	37	11	12	11	16	9	27	46	8
	A_R	25.962	7.856	9.491	8.834	11.322	7.299	21.177	27.861	7.371
	H_o	0.897	0.627	0.669	0.685	0.496	0.661	0.805	0.872	0.756
	H_E	0.958	0.700	0.695	0.793	0.864	0.703	0.945	0.960	0.795
	F_{IS}	0.068	0.109	0.041	0.14	0.43	0.064	0.153	0.096	0.053
2002	**N**	383	374	362	382	381	387	363	340	346
	N_a	37	14	14	13	21	18	31	57	9
	A_R	24.479	9.112	9.323	9.567	12.535	9.878	21.037	27.184	7.079
	H_o	0.822	0.794	0.464	0.518	0.612	0.711	0.686	0.697	0.777
	H_E	0.958	0.776	0.623	0.802	0.876	0.784	0.945	0.958	0.797
	F_{IS}	0.142	-0.022	0.256	0.355	0.303	0.095	0.275	0.274	0.026
2003	**N**	166	169	165	166	163	168	156	134	136
	N_a	35	11	12	14	20	10	28	49	8
	A_R	24.077	7.873	8.153	9.167	12.965	8.133	20.067	28.021	7.368
	H_o	0.873	0.846	0.485	0.699	0.564	0.845	0.673	0.739	0.757
	H_E	0.954	0.789	0.538	0.781	0.881	0.775	0.938	0.961	0.793
	F_{IS}	0.087	-0.069	0.102	0.108	0.362	-0.087	0.286	0.234	0.048

Table 1 cont.

		M1	M3	M6	M7	M8	Ca6	Ca8	Ppro118	Ppro126
2004	**N**	158	156	154	155	159	160	160	131	152
	N_a	35	12	12	14	21	12	27	45	10
	A_R	25.198	9.32	9.912	8.964	15.24	8.829	19.938	26.067	8.1
	H_o	0.835	0.859	0.662	0.645	0.774	0.656	0.863	0.748	0.796
	H_E	0.960	0.813	0.687	0.782	0.892	0.803	0.941	0.952	0.811
	F_{IS}	0.133	-0.054	0.039	0.178	0.136	0.186	0.086	0.218	0.021
2005	**N**	310	349	354	344	309	324	344	371	368
	N_a	38	14	18	16	22	15	30	53	10
	A_R	24.835	11.076	10.869	11.197	13.505	9.673	20.726	27.282	6.987
	H_o	0.910	0.848	0.664	0.648	0.612	0.701	0.828	0.757	0.791
	H_E	0.958	0.859	0.705	0.859	0.896	0.820	0.940	0.958	0.784
	F_{IS}	0.052	0.014	0.059	0.247	0.319	0.147	0.12	0.211	-0.007
2006	**N**	365	379	344	334	356	376	336	368	380
	N_a	42	17	15	16	20	13	30	54	9
	A_R	25.774	11.25	10.162	10.259	13.748	7.954	20.981	27.361	6.977
	H_o	0.959	0.850	0.642	0.677	0.590	0.707	0.792	0.813	0.789
	H_E	0.961	0.851	0.701	0.821	0.894	0.795	0.947	0.959	0.790
	F_{IS}	0.004	0.003	0.085	0.177	0.341	0.111	0.166	0.154	0.002
Cs-01	**N**	56	57	56	56	64	64	57	59	62
	N_a	26	8	10	9	14	10	20	28	7
	A_R	21.689	7	8.589	8.033	11.035	7.829	17.77	20.519	6.863
	H_o	0.821	0.807	0.661	0.625	0.625	0.781	0.491	0.644	0.758
	H_E	0.940	0.746	0.631	0.800	0.782	0.679	0.923	0.902	0.765
	F_{IS}	0.135	-0.073	-0.039	0.228	0.208	-0.142	0.475	0.294	0.018
Cs-An-02	**N**	50	47	51	51	50	51	51	48	46
	N_a	10	12	7	6	10	6	12	22	4
	A_R	8.28	9.489	5.928	5.353	9.13	5.998	9.732	17.803	3.583
	H_o	0.860	0.319	0.412	0.353	0.280	0.843	0.902	0.896	0.783
	H_E	0.785	0.591	0.502	0.652	0.783	0.791	0.808	0.899	0.518
	F_{IS}	-0.085	0.468	0.19	0.467	0.648	-0.056	-0.107	0.014	-0.503
Cs-SA-02	**N**	49	46	52	52	52	53	52	52	52
	N_a	27	9	7	10	14	9	20	27	7
	A_R	22.11	7.858	6.246	8.913	11.556	8.845	17.944	22.164	6.961
	H_o	0.857	0.717	0.423	0.538	0.481	0.792	0.423	0.885	0.846
	H_E	0.936	0.736	0.576	0.810	0.852	0.712	0.929	0.939	0.781
	F_{IS}	0.094	0.036	0.275	0.344	0.444	-0.103	0.551	0.068	-0.074
TFT039	**N**	48	48	42	50	48	51	51	43	40
	N_a	25	11	11	9	12	8	22	26	6
	A_R	20.641	10.162	10.57	8.465	11.394	7.223	18.964	21.908	5.449
	H_o	0.958	0.625	0.643	0.420	0.479	0.549	0.882	0.837	0.750
	H_E	0.929	0.762	0.782	0.791	0.883	0.666	0.918	0.928	0.692
	F_{IS}	-0.021	0.19	0.189	0.477	0.466	0.186	0.049	0.11	-0.07

Table 1 cont.

		M1	M3	M6	M7	M8	Ca6	Ca8	Ppro118	Ppro126
Cs- 04	**N**	47	46	46	45	45	42	47	41	43
	N_a	30	9	10	12	17	10	19	31	7
	A_R	26.228	8.379	8.821	11.474	15.356	8.968	17.968	26.945	6.674
	H_o	0.830	0.696	0.522	0.533	0.689	0.619	0.787	0.854	0.837
	H_E	0.957	0.745	0.740	0.838	0.890	0.791	0.924	0.954	0.812
	F_{IS}	0.144	0.077	0.305	0.373	0.237	0.229	0.159	0.118	-0.02
TFT04-23	**N**	39	42	45	46	33	47	48	42	46
	N_a	23	9	9	8	15	10	16	23	6
	A_R	21.207	8.316	8.535	7.565	14.687	8.466	14.086	20.579	5.998
	H_o	0.846	0.643	0.622	0.457	0.697	0.894	0.958	0.595	0.848
	H_E	0.926	0.698	0.615	0.759	0.865	0.824	0.898	0.930	0.755
	F_{IS}	0.099	0.091	0	0.408	0.209	-0.074	-0.056	0.37	-0.112
TFT04-24	**N**	45	42	43	43	38	34	44	39	43
	N_a	23	11	11	10	12	9	20	18	7
	A_R	20.398	10.335	10.33	8.663	11.449	8.975	18.299	16.379	6.317
	H_o	0.933	0.786	0.977	0.535	0.526	0.824	0.932	0.667	0.744
	H_E	0.930	0.844	0.811	0.800	0.868	0.830	0.921	0.919	0.709
	F_{IS}	0.008	0.081	-0.193	0.342	0.405	0.023	0	0.287	-0.038
TFT04-25	**N**	47	41	42	43	40	46	45	45	50
	N_a	19	10	11	12	12	9	16	21	6
	A_R	16.106	9.638	10.439	10.694	11.357	8.785	13.679	18.255	4.74
	H_o	0.851	0.927	0.833	0.860	0.525	0.696	0.844	0.911	0.940
	H_E	0.897	0.839	0.742	0.870	0.880	0.796	0.867	0.905	0.610
	F_{IS}	0.062	-0.092	-0.111	0.023	0.414	0.136	0.038	0.005	-0.534
TFT04-29	**N**	49	42	46	46	37	47	41	44	29
	N_a	29	13	11	12	13	12	23	30	7
	A_R	23.722	11.979	10.535	9.985	12.511	10.264	18.939	23.845	7
	H_o	0.980	0.786	0.652	0.609	0.676	0.830	0.878	0.750	0.862
	H_E	0.943	0.847	0.720	0.783	0.888	0.837	0.890	0.928	0.786
	F_{IS}	-0.029	0.085	0.105	0.233	0.252	0.02	0.026	0.203	-0.079
TFT04-30	**N**	44	45	50	50	55	54	52	48	42
	N_a	28	12	11	12	14	9	27	29	8
	A_R	24.01	11.015	9.703	10.659	12.062	7.934	21.554	23.823	7.056
	H_o	0.932	0.867	0.680	0.700	0.509	0.722	0.827	0.750	0.833
	H_E	0.938	0.825	0.682	0.823	0.860	0.765	0.930	0.941	0.739
	F_{IS}	0.018	-0.039	0.014	0.159	0.415	0.065	0.121	0.213	-0.116
TFT04-31	**N**	36	46	49	48	41	44	47	46	40
	N_a	25	8	10	13	12	9	21	24	7
	A_R	23.04	7.558	8.601	10.645	11.575	8.655	18.468	20.981	6.377
	H_o	0.833	0.674	0.653	0.625	0.488	0.841	0.787	0.870	0.850
	H_E	0.933	0.724	0.706	0.813	0.880	0.829	0.930	0.936	0.694
	F_{IS}	0.121	0.081	0.085	0.241	0.456	-0.003	0.164	0.082	-0.212

Table 1 cont.

		M1	M3	M6	M7	M8	Ca6	Ca8	Ppro118	Ppro126
TFT05-06	**N**	47	44	47	38	34	45	45	43	43
	N_a	19	8	8	7	12	8	15	16	5
	A_R	16.574	7.647	7.924	6.524	11.771	7.988	13.267	14.581	4.894
	H_o	0.915	0.727	0.702	0.395	0.588	0.733	0.911	0.721	0.512
	H_E	0.922	0.827	0.787	0.747	0.830	0.804	0.860	0.887	0.654
	F_{IS}	0.019	0.132	0.118	0.482	0.305	0.099	-0.048	0.199	0.229
TFT05-07	**N**	37	40	45	40	29	42	42	47	47
	N_a	23	9	11	12	13	8	21	21	7
	A_R	20.847	8.632	10.141	10.803	13	7.288	18.872	17.987	6.423
	H_o	0.892	0.800	0.733	0.675	0.621	0.524	0.905	0.681	0.894
	H_E	0.924	0.826	0.758	0.813	0.850	0.778	0.923	0.878	0.706
	F_{IS}	0.048	0.044	0.044	0.182	0.286	0.337	0.032	0.235	-0.256
TFT05-08	**N**	41	47	45	39	41	46	44	45	48
	N_a	17	9	8	11	12	9	17	20	8
	A_R	16.45	8.878	7.395	10.293	11.669	8.202	15.77	16.844	7.756
	H_o	0.902	0.723	0.689	0.692	0.512	0.804	0.818	0.578	0.708
	H_E	0.924	0.804	0.731	0.814	0.880	0.786	0.882	0.908	0.791
	F_{IS}	0.035	0.11	0.069	0.162	0.428	-0.013	0.084	0.373	0.114
TFT05-09	**N**	39	50	47	47	36	43	45	48	49
	N_a	23	8	8	9	11	8	23	26	7
	A_R	21.138	7.349	7.225	8.322	10.727	7.892	19.939	20.883	6.408
	H_o	0.897	0.660	0.553	0.596	0.556	0.698	0.867	0.896	0.776
	H_E	0.934	0.758	0.635	0.823	0.855	0.756	0.913	0.918	0.716
	F_{IS}	0.052	0.14	0.14	0.286	0.362	0.088	0.062	0.035	-0.074
TFT05-11	**N**	47	47	51	49	44	46	50	46	47
	N_a	24	8	10	11	12	9	18	23	6
	A_R	20.928	7.848	8.584	9.842	10.506	8.18	15.6	19.615	6
	H_o	0.872	0.936	0.569	0.673	0.636	0.652	0.660	0.674	0.787
	H_E	0.938	0.813	0.598	0.843	0.808	0.741	0.881	0.891	0.785
	F_{IS}	0.081	-0.141	0.06	0.211	0.223	0.131	0.26	0.254	0.008
WcE-01	**N**	170	173	162	172	167	176	166	160	163
	N_a	33	14	13	16	20	17	27	51	10
	A_R	23.718	8.921	9.668	9.51	13.201	11.396	19.503	30.607	7.591
	H_o	0.576	0.566	0.463	0.488	0.425	0.642	0.536	0.837	0.785
	H_E	0.955	0.788	0.661	0.800	0.880	0.824	0.940	0.968	0.791
	F_{IS}	0.399	0.284	0.302	0.392	0.519	0.223	0.432	0.137	0.01
WcESA-01	**N**	50	47	48	50	48	46	50	39	42
	N_a	27	9	11	10	12	10	21	31	7
	A_R	22.481	7.812	10.051	9.132	11.139	9.43	18.72	27.859	6.897
	H_o	0.760	0.830	0.542	0.580	0.542	0.804	0.900	0.846	0.810
	H_E	0.939	0.740	0.695	0.776	0.886	0.841	0.931	0.946	0.781
	F_{IS}	0.201	-0.11	0.23	0.262	0.397	0.055	0.043	0.119	-0.025

Table 1 cont.

		M1	M3	M6	M7	M8	Ca6	Ca8	Ppro118	Ppro126
WcE-An-02	**N**	44	50	48	47	50	50	48	45	46
	N_a	22	10	11	8	13	9	23	23	7
	A_R	19.56	8.875	9.199	7.705	11.062	7.554	18.499	19.128	6.855
	H_o	0.955	0.660	0.708	0.489	0.480	0.500	0.750	0.800	0.848
	H_E	0.928	0.801	0.678	0.805	0.800	0.728	0.916	0.915	0.798
	F_{IS}	-0.017	0.185	-0.034	0.401	0.408	0.322	0.192	0.137	-0.052
WcE-SA-02	**N**	79	74	79	76	81	80	62	72	75
	N_a	33	12	12	13	12	11	25	41	7
	A_R	25.307	10.351	9.162	11.408	9.3	9.386	20.286	28.492	6.933
	H_o	0.722	0.527	0.430	0.303	0.444	0.637	0.516	0.847	0.827
	H_E	0.957	0.828	0.724	0.856	0.852	0.745	0.934	0.961	0.801
	F_{IS}	0.252	0.369	0.411	0.65	0.483	0.15	0.454	0.126	-0.026
WcE-03	**N**	51	51	49	51	51	51	51	46	49
	N_a	27	11	12	13	15	9	23	32	8
	A_R	23.598	9.494	10.788	10.67	13.256	8.422	20.313	27.347	7.433
	H_o	0.961	0.784	0.633	0.549	0.647	0.686	0.824	0.587	0.796
	H_E	0.951	0.760	0.719	0.801	0.890	0.776	0.932	0.953	0.737
	F_{IS}	0	-0.022	0.13	0.323	0.282	0.126	0.126	0.393	-0.069

Abbreviations used in samples names: Captive Spawn (Cs), wild-caught eggs (WcE), river reach where eggs were collected: Angostura (An), Isleta (Isl), San Acacia (SA). TFT numbers refer to field number and are all captively spawned lots. Additional details are provided in Turner and Osborne (2005).

Table 2. Summary statistics for microsatellite and mtDNA – ND4 loci for wild (1987, 1999-2006), 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*), allelic richness (A_R) and observed number of haplotypes are given.

	Microsatellites					Mt-DNA			
	N	H_E	H_O	A_R	F_{IS}	N	*h*	A_R	N^o Haps
1987	43	0.818	0.707	-	0.138	37	0.734	6.000	6
1999	46	0.832	0.624	-	0.253	44	0.427	4.968	5
2000	194	0.825	0.718	13.555	0.130	127	0.391	4.956	6
2001	128	0.827	0.719	14.130	0.132	121	0.602	8.200	10
2002	390	0.837	0.676	14.466	0.193	379	0.644	5.834	8
2003	169	0.826	0.720	13.980	0.128	167	0.524	7.056	9
2004	162	0.852	0.760	14.619	0.108	164	0.612	9.449	10
2005	394	0.866	0.751	15.128	0.133	396	0.610	7.892	10
2006	383	0.859	0.758	14.941	0.118	376	0.620	7.630	10
WcE-01	178	0.848	0.591	14.902	0.303	157	0.627	6.999	8
WcE-SA-01	50	0.846	0.735	13.725	0.133	51	0.624	6.000	6
WC-An-02	50	0.827	0.688	12.049	0.170	49	0.481	2.949	3
WcE-SA-02	81	0.856	0.584	14.514	0.320	81	0.702	7.376	8
WcE-SA-03	51	0.844	0.719	14.591	0.150	51	0.714	7.848	8
Cs-01	64	0.803	0.690	12.147	0.142	58	0.460	4.982	5
Cs-An-02	51	0.710	0.627	8.366	0.118	51	0.000	1.000	1
Cs-SA-02	53	0.816	0.663	12.511	0.189	53	0.751	5.919	6
TFT039	51	0.826	0.683	12.753	0.175	51	0.558	3.995	4
Cs- 04	50	0.860	0.707	14.535	0.179	47	0.586	5.911	6
TFT04-23	50	0.817	0.729	12.160	0.110	48	0.593	4.996	5
TFT04-24	48	0.859	0.769	12.349	0.105	48	0.609	4.949	5
TFT04-25	50	0.833	0.821	11.521	0.014	50	0.702	5.934	6
TFT04-29	54	0.857	0.780	14.309	0.091	54	0.609	4.903	5
TFT04-30	56	0.842	0.758	14.202	0.101	55	0.656	4.790	5
TFT04-31	50	0.837	0.736	12.878	0.122	50	0.706	6.865	7
TFT05-06	50	0.823	0.689	10.130	0.164	50	0.625	5.803	6
TFT05-07	49	0.839	0.747	12.666	0.111	49	0.550	4.884	5
TFT05-08	50	0.845	0.714	11.473	0.156	50	0.611	4.934	5
TFT05-09	50	0.821	0.722	12.209	0.122	50	0.506	3.996	4
TFT05-11	51	0.820	0.718	11.900	0.125	51	0.573	5.853	6

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**	**R**	**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
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	-	-		-	-	-	-	-
WC-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	-	-
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	-	-	-	-	-	-	-	0.019	-
Cs-04	0.596	0.255	0.021	-	0.043	0.064	-	-	-	-	-	0.021	-	-
TFT039	0.596	0.269	0.038	-	-	0.096	-	-	-	-	-	-	-	-
TFT04-23	0.617	0.043	0.191	-		0.043	-	-	-	-	-	0.106	-	-
TFT04-24	0.583	0.125	0.208	-	0.021	0.063	-	-	-	-	-	-	-	-
TFT04-25	0.434	0.057	0.113	0.057	0.283	0.057	-	-	-	-	-	-	-	-
TFT04-29	0.566	0.245	-	0.075	-	0.094	-	-	0.019	-	-	-	-	-
TFT04-30	0.400	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	-	-	-	-	-

Table 4. a) Pairwise F_{STs} calculated from microatellite data among wild, temporal samples (below diagonal) and P-values (above diagonal). **b**) Pairwise θ_{STs} calculated from Mt-DNA data among wild, temporal samples (below diagonal) and P-values (above diagonal). Significant values (after Bonferroni correction) are shaded.

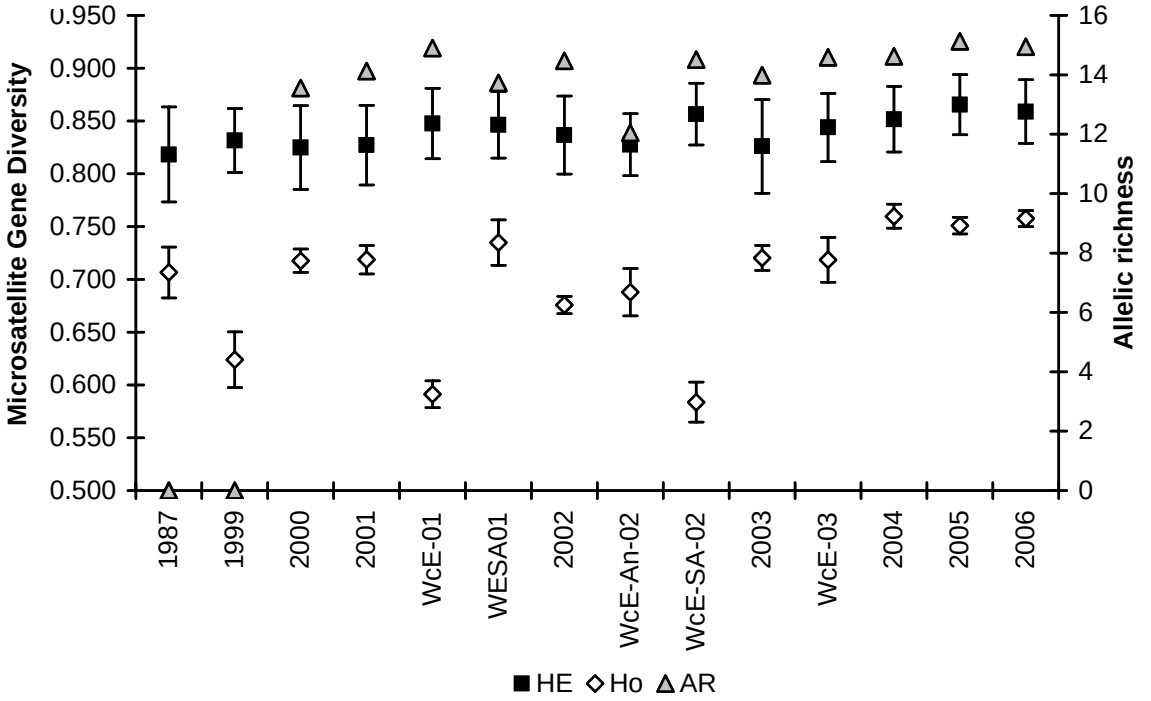
a.

usats	1987	1999	2000	2001	2002	2003	2004	2005	2006
1987	-	0.9998	0.5941	0.4737	0.0008	0.0000	0.0000	0.0000	0.0000
1999	-0.0272	-	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998
2000	0.0001	-0.0226	-	0.0181	0.0000	0.0000	0.0000	0.0000	0.0000
2001	0.0007	-0.0261	0.0019	-	0.0006	0.0000	0.0000	0.0000	0.0000
2002	0.0072	-0.0298	0.0044	0.0027	-	0.6646	0.0000	0.9998	0.0000
2003	0.0109	-0.0308	0.0053	0.0047	0.0000	-	0.0002	0.9996	0.0000
2004	0.0205	-0.0300	0.0147	0.0139	0.0042	0.0044	-	0.9998	0.0145
2005	0.0074	-0.0167	0.0062	0.0053	-0.0023	-0.0025	-0.0046	-	0.7899
2006	0.0219	-0.0166	0.0184	0.0179	0.0073	0.0054	0.0013	-0.0002	-

b.

Nd4	1987	1999	2000	2001	2002	2003	2004	2005	2006
1987	-	0.1412	0.0069	0.0075	0.2654	0.1891	0.0518	0.0302	0.1040
1999	0.0201	-	0.3848	0.2434	0.0817	0.6765	0.4854	0.2920	0.3359
2000	0.0778	-0.0017	-	0.2617	0.0000	0.0304	0.0663	0.0266	0.0075
2001	0.0589	0.0044	0.0021	-	0.0020	0.0403	0.3688	0.2016	0.0490
2002	0.0029	0.0183	0.0492	0.0295	-	0.0528	0.0302	0.0040	0.0429
2003	0.0096	-0.0088	0.0192	0.0159	0.0083	-	0.3063	0.0962	0.2160
2004	0.0274	-0.0042	0.0105	-0.0002	0.0107	0.0005	-	0.7247	0.4803
2005	0.0308	0.0019	0.0120	0.0022	0.0122	0.0051	-0.0023	-	0.1857
2006	0.0156	-0.0006	0.0208	0.0094	0.0053	0.0015	-0.0010	0.0015	-

1a.



1b.

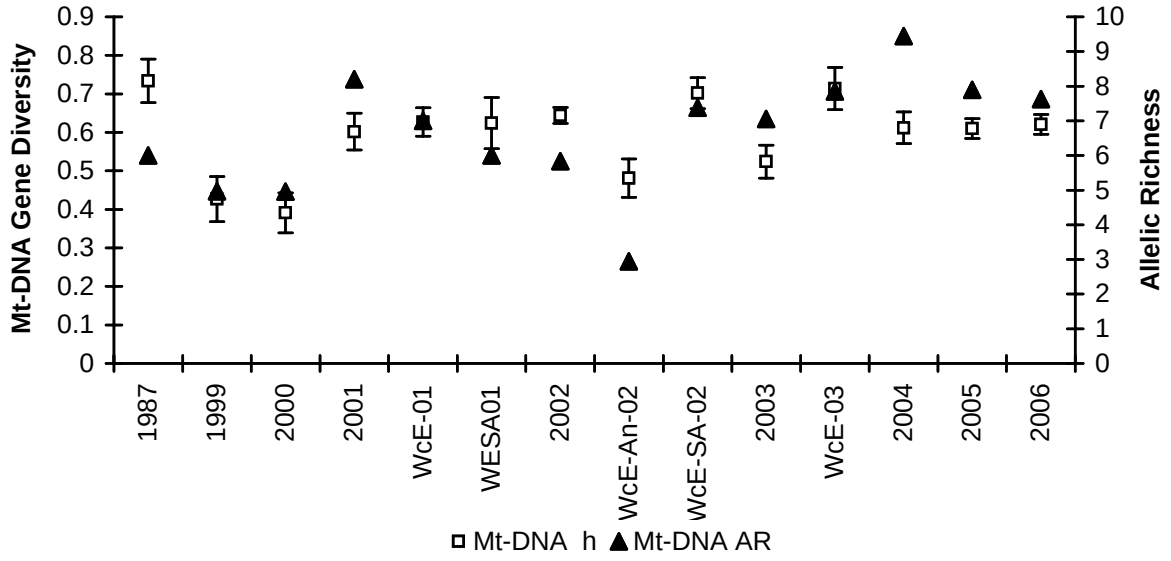


Figure 1. Diversity statistics for wild and stocks from wild-caught eggs. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (h) and allelic richness are given (**b**). Standard deviation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

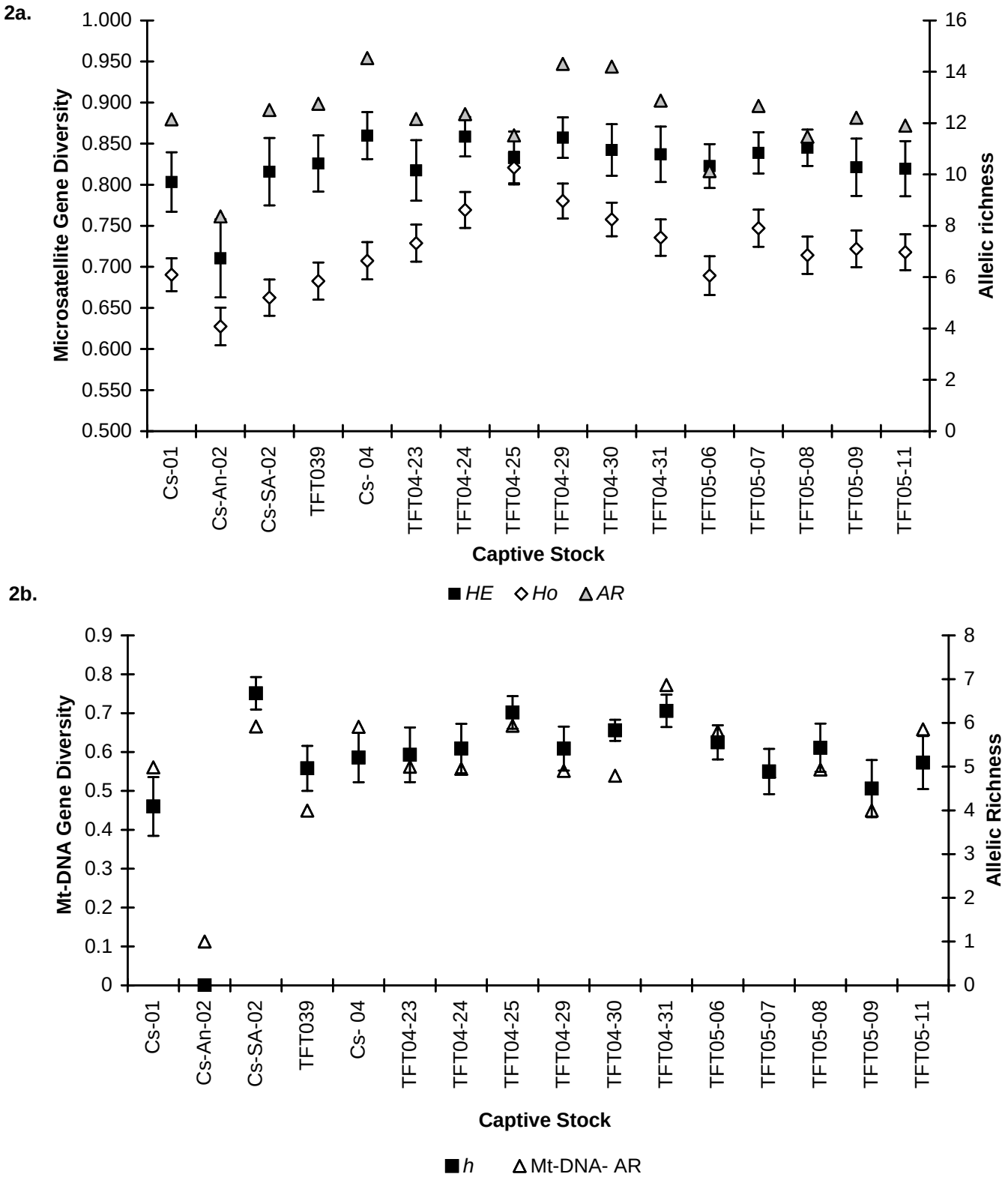


Figure 2. Diversity statistics for captive stocks. For microsatellites expected heterozygosity (H_E), observed heterozygosity (H_O) and alleilc richness (A_R) are shown (**a**). For mitochondrial DNA gene diversity (*h*) and allelic richness are given (**b**). Standard deviation bars are given for *h*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

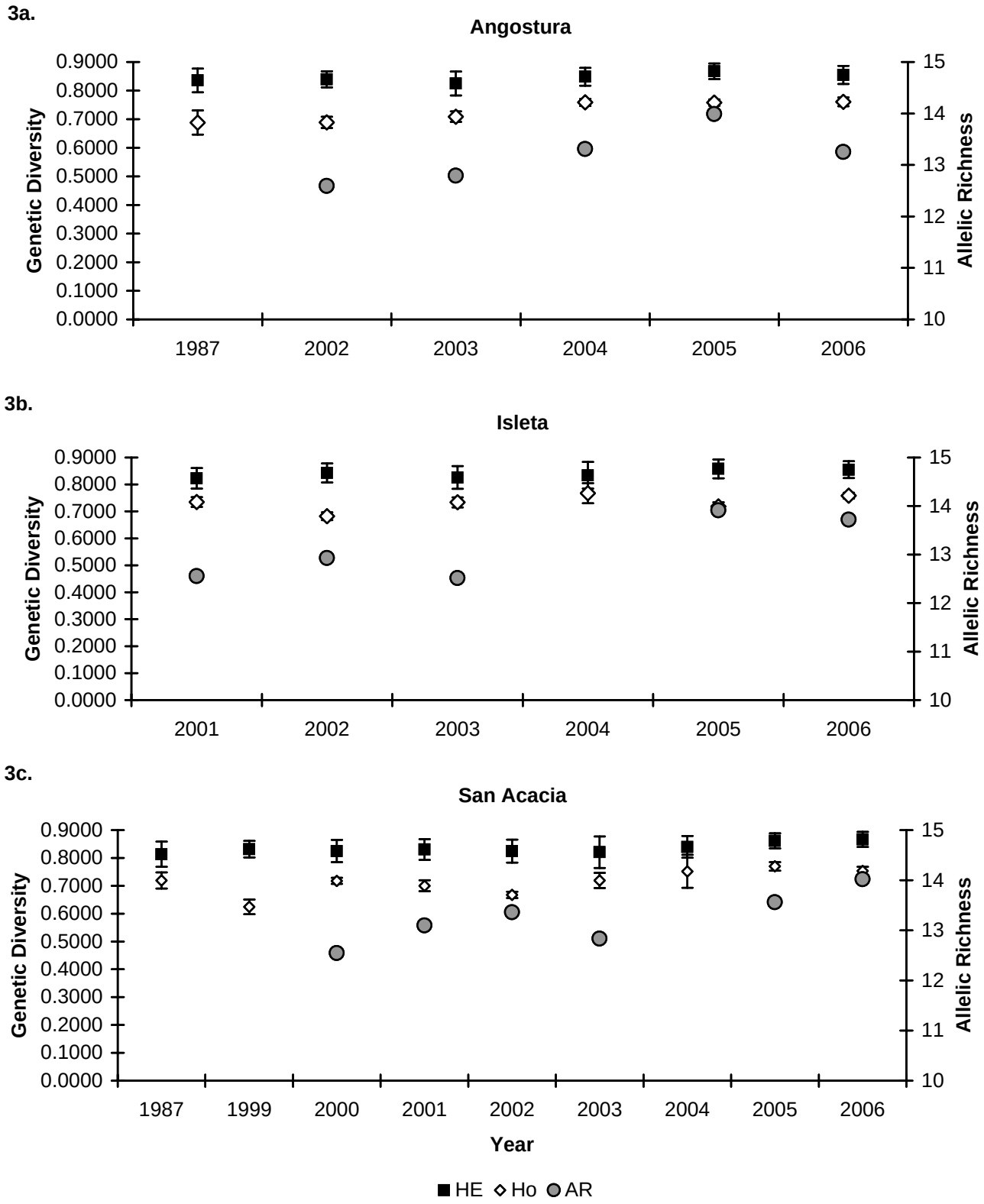


Figure 3. Diversity statistics by river reach a) Angostura, b) Isleta, c) San Acacia and year from microsatellite DNA data. Expected heterozygosity (H_E), observed heterozygosity (H_O) and allelic richness are given. Standard deviation bars are given for H_E and H_O.

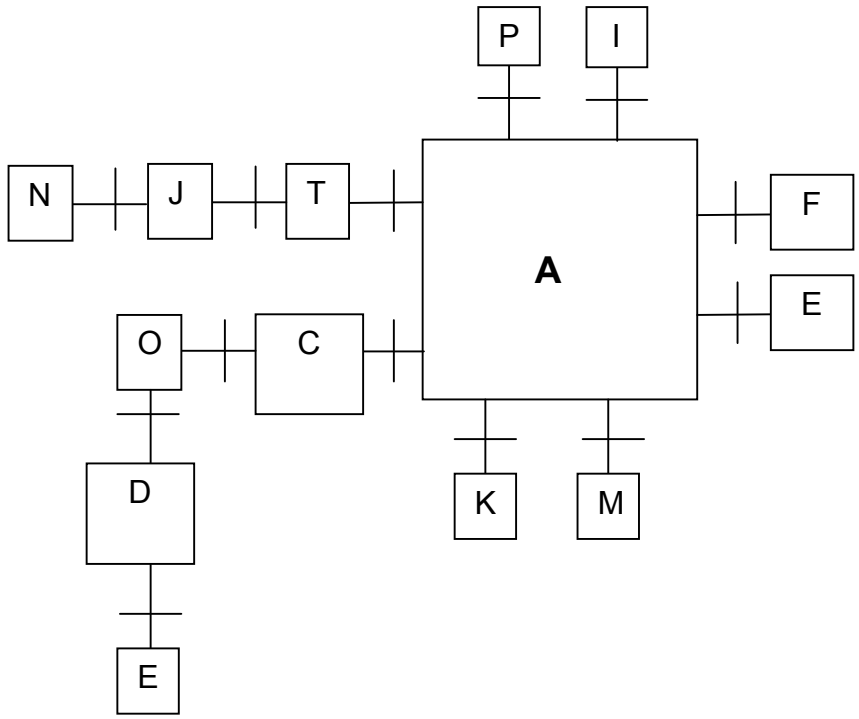


Figure 4. Statistical parsimony network of mitochondrial DNA haplotypes identified in Rio Grande silvery minnow. Each bar indicates a single nucleotide change. Box sizes reflect the frequencies at which haplotypes were present.

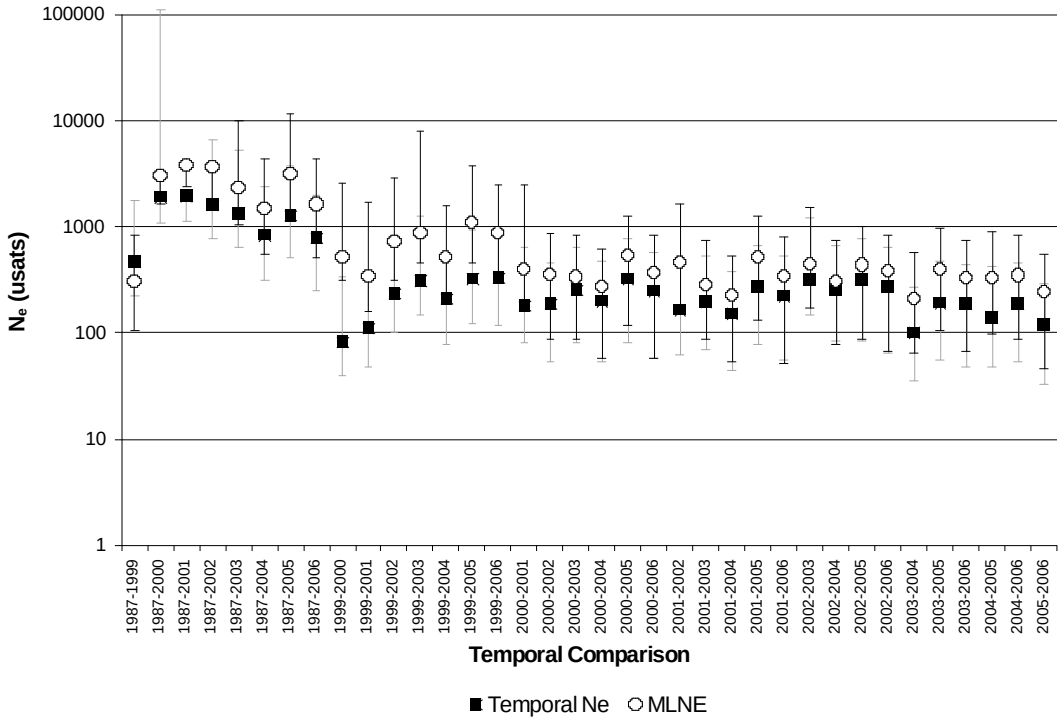


Figure 5. Pairwise estimates of effective population size calculated using the temporal and maximum likelihood methods from microsatellite data. Upper and lower 95% confidence intervals are given for estimates. For 1987-2000, 1987-2001 and 1987-2002 comparisons the upper 95% CIs are infinity.

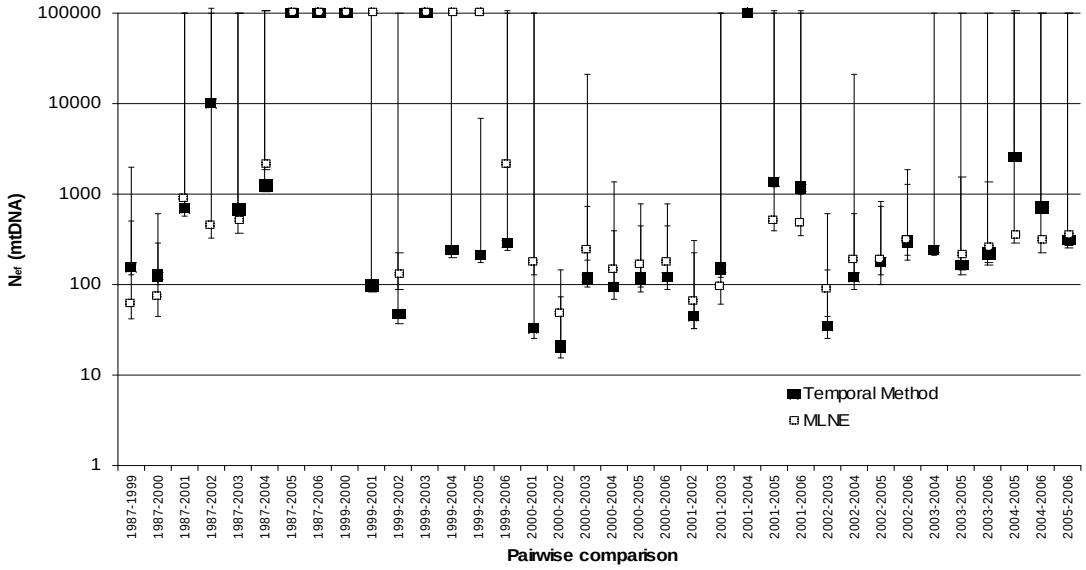


Figure 6. Pairwise estimates of female effective population size calculated using the temporal and maximum likelihood methods from Mt-DNA data. Upper and lower 95% confidence intervals are given for estimates.

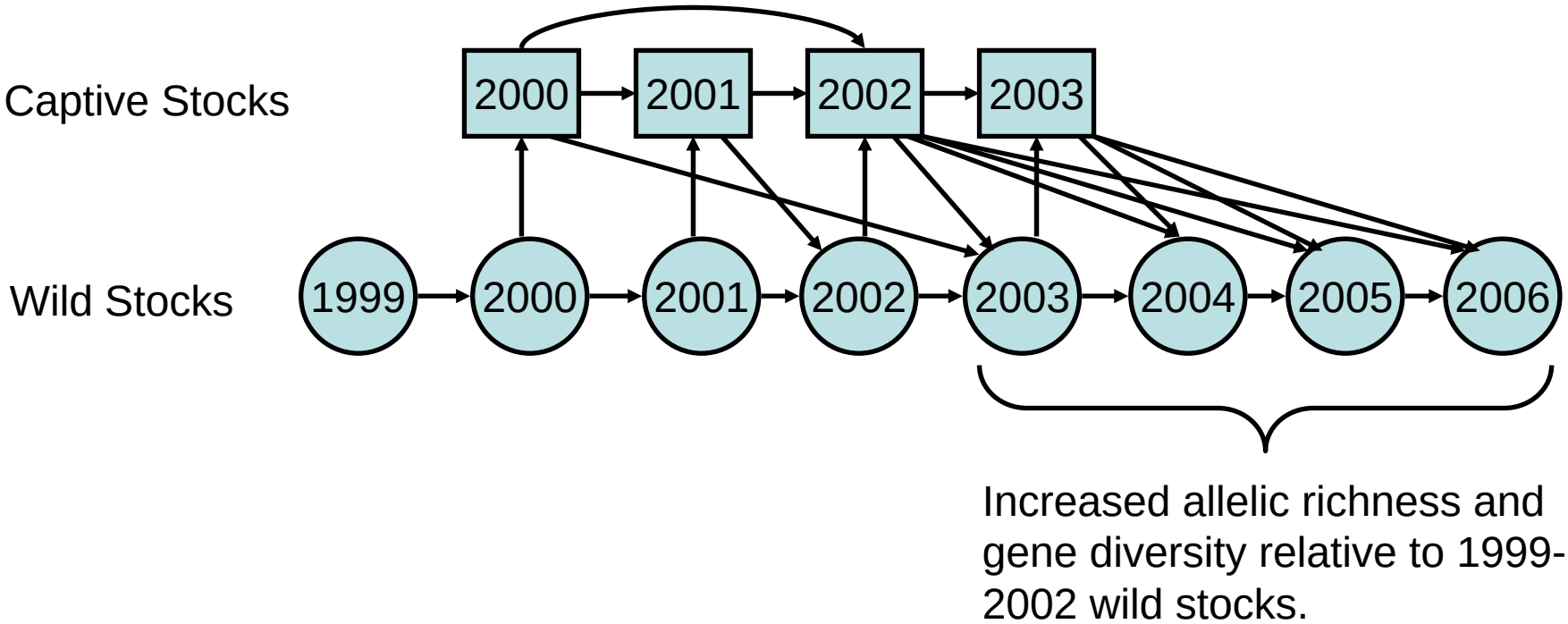


Figure 7. Conceptual representation of multi-year contributions of captive to wild stocks and vise versa. Arrows indicate the direction of transfer of individuals.