Genetic consequences of supportive breeding in the endangered Rio Grande silvery minnow (Hybognathus amarus): genetic evaluation of wild and captively reared and propagated stocks, 1999-2004.

An Annual report for FY 2003 to the US Bureau of Reclamation and Middle Rio Grande Endangered Species Collaborative Workgroup

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Executive Summary

The genetic effects of artificial propagation and captive-rearing of wild-caught Rio Grande silvery minnow eggs were evaluated. The primary aim of this study was to determine whether these two methods of supportive breeding captured the genetic variation and levels of heterozygosity that were present in the remaining 'wild' Rio Grande silvery minnow population. This aim was addressed by examining variation at ten microsatellite loci and a mitochondrial DNA locus for seven generations of wild Rio Grande silvery minnow, five stocks of captively-spawned and reared fish and five stocks of adults reared from wild-caught eggs. The principle findings of this study were:

i) Two dramatic losses of allelic diversity have occurred in the 'wild' (i.e., sampled in the Rio Grande proper) Rio Grande silvery minnow population. These losses followed major declines in abundance of Rio Grande silvery minnow in the wild.

ii) A supportive breeding program that uses wild caught-eggs better reflects the levels of genetic diversity than one that relies on captive spawning. Supplementation of the wild population with adults reared from salvaged eggs appears to have slowed the loss of allelic diversity in the wild population. However, heterozygosity has declined in stocks reared from wild-caught eggs.

iii) The genetic effects of captive spawning are determined primarily by the breeding strategy that is used. The combination of communal spawning (where there is the potential for unequal parental contributions), small broodstocks and unequal sex ratios has resulted in loss of allelic diversity in these stocks compared to wild fish or fish reared from wild-caught eggs. There has also been a slight decline in observed heterozygosity when compared to the wild population. Collaborative work with ABQ Biopark in FY 2004 will compare genetic diversity of progeny from one to one paired matings and communal spawns to identify the optimal strategy from a genetic perspective.

Introduction

Historically, the ichthyofaunal community of the New Mexico portion of the Rio Grande included five species of Great Plains cyprinids. River fragmentation by impoundments and water diversion structures has been particularly devastating for this group of native fishes which are members of the pelagic spawning minnow guild (Platania and Altenbach 1998). Of these species, two are now extinct (*Notropis simus simus*, *Notropis orca*) and two have been extirpated (*Macrhybopsis*, *aestivalis*, *Notropis jemezanus*). The remaining species, the Rio Grande silvery minnow (*Hybognathus amarus*) currently exists as a remnant population and was listed as federally endangered in 1994 (U.S. Fish and Wildlife Service, Federal Register 1994) due to its extirpation from the majority of its historical range. Population monitoring, that began in 1987 and continues to the present (Platania 1993; Dudley et al. 2004), has indicated that the remnant wild population of the Rio Grande silvery minnow is still declining, despite its endangered status. Relative abundance of Rio Grande silvery minnow was around 50% of the total ichthyofaunal community in 1995 and only about 0.5 % in 2003 (Dudley et al. 2004). The continued decline of the Rio Grande silvery minnow is presumably a consequence of habitat degradation, river fragmentation that prevents upstream movement of fishes and extensive dewatering during a recent prolonged drought (Bestgen and Platania 1991).

Conservation of endangered species particularly fishes, often relies heavily on captive propagation and supplementation to aid species recovery and prevent extinction (Waples 1999, Hedrick et al. 2000). If used in conjunction with measures that address the underlying causes of species decline, supportive breeding, augmentation of existing populations and reintroductions of a species into former areas of their geographic range can be successful at recovering endangered species (e.g., Gila Trout – Wares et al. 2004).

Unlike mammals and birds, most fishes are highly fecund but suffer extensive mortality in egg and larval life-stages (McGurk 1986; Houde 1989; Pepin 1991; Bradford & Cabana 1997). This life history presents an opportunity to increase numbers by improving the survivorship of these stages. For Rio Grande silvery minnow, mortality imposed by predation, resource limitation, and catastrophic events can be decreased by either breeding wild-caught adults in captivity or by collecting eggs from the wild and rearing the eggs and larval fish in captivity to the juvenile or adult stage. Once grown to sufficient size, fish are released to supplement the ‘wild’ population.

To date, the majority of fish for augmentation/supplementation in the middle Rio Grande have been produced by these two methods of supportive breeding.

An alternative to captive breeding is collection and rearing of eggs from natural spawning events. In the wild, Rio Grande silvery minnow spawn in response to increases in flow associated with spring runoff and summer rainstorm events (Platania & Altenbach 1996) so the majority of spawning effort occurs over a three to five day time period (Platania & Altenbach 1998). Following spawning, pelagic eggs and larvae are transported downstream by river currents (Platania & Altenbach 1998; R. Dudley Pers. Comm.). This makes it relatively easy to collect a large number of eggs that would otherwise be lost when they are swept into unsuitable nursery habitat (such as Elephant Butte reservoir or reaches of the Rio Grande that are subject to dewatering events in the summer). If enough adults remain in the river to successfully spawn, eggs collected should include the reproductive output of many breeders and represent the genetic diversity that is present in the wild population. Egg capture and subsequent hatchery rearing can minimize the opportunity for genetic adaptation to captivity. As such, many of the problems that can be encountered with a conservation program that relies on captive spawning and rearing, can be avoided. However, if environmental conditions are unable to support a population of sufficient size to breed successfully, then collection of eggs for supportive breeding will be impossible (as seen in 2004).

Captive spawning uses wild or hatchery-reared adult fish as broodstock. Generally, these fish are artificially induced to spawn and their offspring reared in captivity, ultimately to supplement the wild population. There are risks to genetic composition and diversity associated with captive spawning: 1) loss of genetic diversity, 2) inbreeding depression, 3) accumulation of new mildly-deleterious alleles, and 4) genetic adaptation to captivity (Frankham et al. 2000; Lynch and O'Hely 2001). A well-designed broodstock management plan can diminish, but never completely eliminate these risks (Waples 1999). The first three risks relate to small population size. In small populations genetic diversity is eroded over time at a rate roughly the inverse of effective population size (N_e). This process is referred to as genetic drift in which allele frequencies fluctuate due to random sampling of genes during transmission between generations. This process is more extreme when only a limited subset of genes is represented in the population, for example, when a small broodstock is used to generate a captive stock. The depletion of a population's genetic diversity leads to an increase in homozygosity of individuals in the population. This may cause reduced viability and fecundity (inbreeding depression)

(Falconer 1981; Ralls & Ballou 1983). A 10 % loss of genetic diversity has harmful effects on traits such as survival and growth rate (Falconer 1981). Studies on a range of fish species demonstrate that a 25% loss of heterozygosity due to inbreeding is correlated with an increase in morphological deformities and reduced survival of advanced larval and early juvenile stages (Kincaid 1983, Kincaid 1976 a,b). In addition to genetic changes, there may be detrimental behavioral changes that may reduce the fitness of the hatchery-reared individuals once they are returned to the wild (e.g. Hindar et al. 1991). Both supportive breeding and the use of captively-spawned fish for supplementation can result in a reduction in the effective population size of the wild population. This may occur in cases where a large number of fish produced from a few breeders are successfully introduced into the wild (Ryman & Laike 1991; Waples 1991; Waples & Do 1994). 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 is little empirical genetic data on non-salmonid species to examine whether these predictions are realized.

Experimental captive spawning of Rio Grande silvery minnow was commenced in 2000 (Platania and Dudley 2001). In May 2001 Rio Grande silvery minnow eggs were collected from San Marcial for the purpose of captive rearing and eventual release. Eggs have been collected each year since this time for propagation activities. In 2004, a peak of spawning activity was not observed and no eggs were collected for captive rearing (J. Remshardt, USFWS Pers. Comm.). The purpose of this study was to examine the genetic effects of supplementation on the wild population and to consider the genetic consequences of captive rearing and spawning. Results and interpretation are designed to provide recommendations to managers charged with guiding recovery efforts for the Rio Grande silvery minnow.

Background and Experimental Methods

Stocking History

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 a refugial population. Between May and June 2000 eight groups of silvery minnow were artificially induced to spawn (broodstock collected from the San Acacia reach, N = 522) (Platania and Dudley 2001). Larval fish from these efforts were released at Bernalillo (91,600) and Los Lunas (112,000) (Table 1). In 2002, 12,900 Rio Grande silvery minnow were released

in the San Acacia reach of the middle Rio Grande, New Mexico by the University of New Mexico and Museum of Southwestern Biology. Experimental augmentation by U. S. Fish and Wildlife Service Fishery Resource Office began in June 2002 with the release of 2082 adult fishes in the Angostura reach (Alameda Bridge) followed by further releases in December 2002 and January 2003 (103,639 fish) and in April 2003 (22,266 fish) (Davenport and Brooks 2003). In January and April 2004, a further 115,157 fish were released (Remshardt, Pers. Comm.). Between 2000 and 2004 over 400,000 captively reared and/or spawned fish have been released in the middle Rio Grande. All fish released were marked with visible implant elastomer (VIE) tags (2002) or calcein (all fish released in 2004). A portion of calcein marked fish were also VIE tagged. Marking allowed all hatchery-reared fish to be distinguished from wild individuals.

Sampling- Rio Grande Population ('Wild')

Wild Rio Grande silvery minnow populations were sampled each year from 1999 to 2004 (between Dec. and March). We also genotyped 43 individuals used in an allozyme study of *Hybognathus* (MSB Catalogue Number 4636, Cook et al. 1992). In this case "wild" means fish sampled from the Rio Grande proper, but does not imply that the sampled fish was not of hatchery origin at some time in the past. Samples were collected throughout the current range of the species which is located in the Rio Grande between Cochiti and Elephant Butte Reservior, New Mexico, known as the middle Rio Grande (Figure 1). It is fragmented by three water diversion structures which divide the river into four distinct reaches: 1) Cochiti, 2) Angostura, 3) Isleta, and 4) San Acacia. These river segments are 35.9, 65.2, 85.5 and 90.4 kilometers long, respectively. 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 in MS-222 (Tricaine methane sulfonate 200 mg/L river water) at the site of capture and a small piece of caudal fin was removed from each individual. Fin clips were stored in 95% ethanol. Fishes were placed in untreated water to recover prior to release.

Sampling- Wild-caught eggs and captively-spawned stocks

Fish for supplementation purposes came from two primary sources: wild-caught eggs reared to adult size in captivity (roughly 50 mm), and captive spawning and rearing. Four consecutive year classes (2001 to 2004) reared from wild-caught eggs were considered for genetic analysis. The egg salvage site was located approximately 16 kilometers downstream of the San Marcial railroad bridge crossing in the San Acacia reach of the middle Rio Grande, New Mexico. In

2002, eggs were also collected from the Angostura reach. Eggs were collected using modified Moore egg collectors (Altenbach et al. 2000), transported to propagation facilities and reared to adult size.

Five groups of captively spawned fish were considered. Spawning was induced by hormone injection. In one case, spawning occurred in an artificial refugium (Albuquerque Biological Park) without the use of hormone injections (C. Altenbach, Pers. Comm.). Fin clips were taken from adult fish prior to repatriation back to the middle Rio Grande (see stocking history section above). Almost no mortality resulted from anesthesia and fin-clipping in captive adults (M. Osborne, pers. observation).

Molecular Methods- Microsatellites

Total nucleic acids, including genomic and mitochondrial DNA were extracted from air-dried fin clips using proteinase-K digestion and organic extraction methods (Hillis et al. 1996). DNA was extracted from developing eggs (Eggs-03) by mechanically rupturing the egg and resuspending them in 25µL of distilled water. Individuals were genotyped for ten microsatellite loci: *Lco1*, *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7*, *Lco8* (Turner et al. 2004) and *Ca1*, *Ca6* and *Ca8* (Dimsoski et al. 2000). Microsatellites (*Lco1-8*) were amplified and visualized according to the protocols described in Alò and Turner (In Press). *Ca1* and *Ca6* were amplified using multiplex PCR (1X PCR buffer, 2mM $MgCl_2$, 125µM dNTPs, 0.40µM each primer, 0.375 units TAQ polymerase). *Ca8* was amplified alone (1X PCR buffer, 2.5mM $MgCl_2$, 125µM dNTPs, 0.50µM each primer, 0.375 units TAQ polymerase). PCR cycling conditions were: one denaturation cycle of 94°C for 2 mins followed by 30 cycles of 94 °C for 20s, 48°C (*Ca1*, *Ca6*) or 52°C (*Ca8*) for 20 sec, 72°C for 30s. Seven microsatellite loci (*Lco1*, *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7*, *Ca6*) were amplified for the Eggs-03 population because of the limited amount of DNA that could be extracted from these samples.

MtDNA-ND4

Individuals were screened for variation in a 295 base pair fragment of the mitochondrial ND4 gene using SSCP analysis and DNA sequencing as described in Alò and Turner (In Press).

Data Analysis

Allele frequencies, allelic richness, average inbreeding coefficients (F_{IS}), and gene diversity estimates (Nei 1987) were obtained using FSTAT version 2.9.3.1 (Goudet 1995). Allelic richness was calculated using the methods described Petit et al. (1998) which allows the number of alleles to be compared among populations independently of sample size. FSTAT was also used to determine whether allelic richness, H_O, H_E and F_{IS} differed significantly between wild, hatchery-reared wild-caught eggs and captively-spawned stocks. Markov chain methods of Guo and Thompson (1992), as implemented in GENEPOP Version 3.1.d (Raymond and Rousset 1995), were used to determine if genotype frequencies differed significantly from Hardy-Weinberg expectations. Global tests for linkage disequilibrium were conducted for all pairs of loci using GENEPOP. Weir and Cockerham's (1984) *F*-statistics were calculated using ARLEQUIN (Schneider et al. 2000) to determine the magnitude of divergence between the wild source populations and the captively-reared populations.

Results

Genetic Variation- Microsatellites

A total of 2158 samples were characterized using between seven and 10 microsatellite loci. *Lco1* and *Ca8* were most variable with between 10 and 44 alleles observed at *Lco1* and from eight to 31 at *Ca8* (Table 2). Allelic richness (based on the minimum sample size of 39 individuals) in the wild population ranged from 9.000 (1999) to 16.493 (2000) (Table 3, Figure 2). The average number of alleles per locus was highest in the Isleta and San Acacia reaches in 2002 and in the Angostura reach in 2004 (Table 4). Allelic richness in captively-spawned populations ranged from 6.760 (Cs-An-02) to 12.106 (Cs-Dx-04) and for hatchery reared wild-caught eggs was between 10.279 (WcE-An-02) to 12.628 (WcE-Dx-01). Allelic richness was significantly lower in the captively-spawned fish when compared to the wild populations (P = 0.045). However, allelic richness did not differ between wild fishes and captively-reared wild-caught eggs. When wild populations were considered, observed heterozygosity (microsatellite loci) was highest in the year 2000 (Figure 3). Observed heterozygosity was highest in 2003 for wild-caught eggs (Figure 4). Average gene diversity (H_E) and observed heterozygosity (H_O) did not differ significantly between wild, captively spawned and fish reared from wild caught eggs.

Departures from Hardy-Weinberg expectations were observed in nearly all samples (Table 2), and generally resulted from a deficiency of heterozygotes. For *Lco3* (Wild 2002), *Lco4* (Wild

2003) and *Lco5* (Wild 1987, WcE-Dx-01, WcE-SA-02, WcE-SA-03 and Cs-TFT-039) there was an excess of heterozygotes. The range of average weighted inbreeding coefficients (F_{IS}) across all loci was 0.051 (Wild 2003) and 0.268 (Wild 1999) for wild populations, from 0.175 (WcE-SA-03) to 0.410 (WcE-SA-02) for the hatchery-reared wild-caught eggs and between 0.200 and 0.242 for captively-spawned populations (Table 2). The inbreeding coefficient observed in the egg sample (Eggs-03- F_{IS} = 0.084) is lower than that observed in the adults reared from wild-caught eggs (WcE-SA-03 F_{IS} = 0.135) (these eggs were collected at the same place and time). A global test for linkage disequilibrium revealed two instances of linkage disequilibrium (*Lco3* and *Lco4*, *Lco6* and *Ca8*) after Bonferroni correction for multiple hits.

MtDNA-ND4

SSCP analysis and direct sequencing revealed 14 haplotypes in 2125 genetic samples. There were between one and six nucleotide differences between haplotypes. Haplotype A was the most common in all populations with the exception of population Cs-An-02 which was monomorphic for haplotype D. Haplotype A was present at a frequency of between 0.434 and 0.762 (Table 5). Five haplotypes (C, D, E, F, K) were observed at moderate frequencies. Six haplotypes (I, J, N, P, R, S) were observed at a frequency of less than 0.02. In the 2004 wild population 10 haplotypes were observed. The fewest haplotypes were observed in the captively-spawned populations with between one and six haplotypes. Among captively-spawned fish Cs-SA-02 had the highest ND4 diversity (Figure 5).

Population Divergence

Pairwise F_{ST} values (calculated from microsatellite data) were significantly different between all of the populations (Table 6). The average pairwise F_{ST} among wild populations was 0.0260, among wild-caught egg populations was 0.0244 and between captively spawned populations was 0.0656. Average pairwise F_{ST} between the wild populations and hatchery reared wild-caught eggs was 0.0267. The average F_{ST} between the wild and the captively-spawned populations was 0.0465 and between captively spawned and wild-caught eggs was F_{ST} was 0.0401. F_{ST} was 0.0397 between Eggs-03 and WcE-SA-03 and 0.0269 between Eggs-03 and Wild 2004. F_{ST} was 0.0096 (calculated using data from seven microsatellite loci) and 0.0317 (calculated using data from ten microsatellite loci) between WcE-SA-03 and Wild 2004 (Table 7a-d and Figures 6 through 8).

Discussion

We assessed the impacts of two primary risks associated with small population size (loss of genetic diversity, loss of heterozygosity) in wild, artificially spawned and captively-reared populations of Rio Grande silvery minnow.

Genetic Diversity- Wild Population

In small populations the overriding genetic process affecting the population is the erosion of diversity due to genetic drift (Lacy 1987). Loss of genetic diversity limits a species or populations ability to adapt and respond to environmental changes and can increase susceptibility to extinction (Frankham 1995; Higgins and Lynch 2001). Data presented here indicates that there has been two sharp declines in allelic diversity in the 'wild' population, the first occurred in 1999 and the second, in 2001. Both losses of diversity followed sharp declines in abundance (catch rates declined by an order of magnitude) of Rio Grande silvery minnow between 1995 and 1997 and between 1999 and 2000 (Dudley et al. 2004), which are concomitant with extensive river drying in the San Acacia Reach of the Rio Grande. Although the wild population has continued to decline drastically since 2001 reaching extremely low levels in 2003, there has not been a substantial loss of allelic diversity over this time period. Supplemental stocking with captively-reared wild caught-eggs between 2001 and 2003 may have slowed or temporarily alleviated loss of alleles in the wild. However, the continued decline of the wild Rio Grande silvery minnow population will result in erosion of its genetic diversity and will translates into a loss of diversity in the hatchery-reared wild-caught eggs and hence, in the adult fish available for supplementation.

Genetic Diversity- Captively-reared wild-caught eggs

Allelic diversity (microsatellites) is retained in the captively-reared wild-caught eggs at relatively constant levels between years (Table 2). Wild-caught eggs collected in the Angostura reach in 2002 (WcE-An-02) are an exception, with less diversity present for both microsatellite and ND4 loci. There is a downstream increase in allelic diversity (average number of alleles per locus) in 2001 and 2002 with a greater number of alleles present in the San Acacia reach when compared to the Isleta and Angostura reaches. This trend is reversed in 2003 and 2004 and may reflect adult mortality and/or poor recruitment resulting from extensive dewatering of the Isleta and San Acacia reaches in 2002 and 2003. Dewatering of the southern reaches of the middle Rio Grande

greatly limits or eliminates appropriate nursery habitat that larval fish require for development and survival (Pease 2004).

Genetic Diversity- Captively propagated stocks

Our findings indicate that genetic diversity present in artificially spawned stocks is dependent on the number of broodstock that were used, as expected from theory. Two stocks (Cs-SA-02 and Cs-An-02) are the progeny of small broodstock number (Table 1) and an unequal sex ratio. Effects of genetic drift will be extreme when small broodstocks are used, and could represent an extreme population bottleneck resulting in loss of alleles from subsequent generations if progeny are used to repopulate wild stocks. Larger broodstocks (but unequal sex ratios) were used to produce the 2004 (Cs-Dx-04) captive stocks resulting in levels of allelic diversity equivalent to those observed in the wild population. The captive stock (TFT-039) produced in the Albuquerque Biological Park artificial refugium (without the use of hormone injections) retained similar levels of allelic diversity to the parental stock (WcE-SA-01), but loss of alleles was evident in both the parental stock and F1 generation (TFT-039) when compared to the wild source population (Wild 2001). Alleles are lost due to genetic drift at a rate that depends on their frequencies in the population with alleles present at high frequencies likely to persist longer in the population than rare alleles.

Heterozygosity: Wild population

The depletion of a population's genetic diversity leads to an increase in the number of homozygous individuals in the population (Lacy 1987). This can lead to inbreeding depression (reduced fecundity and survivorship) in the population. Heterozygosity is expected to be lost at a slower rate than allelic diversity (Allendorf and Ryman 1987) so even small populations retain most of the original population's heterozygosity. Unfortunately, we do not have data that predates the decline of the Rio Grande silvery minnow with which to compare the post-decline data (the species was extirpated from the majority of its range by 1979- Bestgen & Platania 1991; Propst et al. 1987; Bestgen et al. 1989; Edwards & Contreras-Balderas 1991).

Heterozygosity: Wild-caught eggs and captively spawned stocks

Adults reared from wild-caught eggs retain between 84 % and 95 % of the heterozygosity of parental stock and the captively spawned stocks retain between 79 % and 96 % of the parental population's observed heterozygosity. For two populations (Cs-An-02 and Cs-SA-02) broodstock information is available so we can estimate how much heterozygosity is expected to

be retained using (1-1/N) (where N is the size of the bottleneck) (Allendorf and Ryman 1987). For Cs-SA-02 the observed heterozygosity retained between the parental population and the progeny is close to that predicted (95.6% compared to 97.8%) but for the Cs-An-02 stock is less than predicted (79% compared to 96.6%). The difference between the predicted and observed values can be explained by variance in reproductive success in which not all potential parents in the broodstock contributed equally. The mitochondrial DNA data support this with only a single ND4 haplotype observed. This suggests that a single female may have contributed the majority of the offspring. In both captively reared wild-caught eggs and artificially spawned stocks the average weighted inbreeding coefficients are higher on average than in the wild population.

Management recommendations based on our findings

1. A supportive breeding program based on eggs collected from the wild and reared in captivity is preferable from a genetic perspective than a program that relies solely on captive spawning of wild or F1 generation fish. Wild-caught eggs reflect the genetic diversity that is observed in the wild population and reduces the potential for domestication selection and/ or relaxation of selection that may occur in captivity. Results indicate that the introduction of individuals reared from wild-caught eggs has slowed the depletion of allelic diversity in the 'wild' population. There are several other advantages of using wild-caught eggs including a) avoiding manipulation and potential mortality of adult fish that may occur during artificial spawning, and b) not removing wild adult fish from the population for broodstock purposes. However, this strategy depends entirely on the spawning success of the wild population. As such, river conditions, particularly flows and population numbers must be adequate to enable Rio Grande silvery minnow to successfully spawn and produce sufficient numbers of eggs for efficient salvage. We recommend future egg salvage efforts from the middle Rio Grande for the reasons outlined above. The continued decline of the wild population and increased reliance on captive spawning to produce Rio Grande silvery minnow for augmentation purposes is likely to result in further losses of genetic diversity in the middle Rio Grande population.

2. A program of captive spawning should also be implemented because the use of wild-caught eggs depends solely on the reproductive success of the wild population. Captive spawning and development of a broodstock management plan provides some level of insurance in the event of reproductive or recruitment failure in any year. Risks to overall levels of genetic diversity can be minimized or amplified depending on the breeding strategies that are implemented. Our findings indicate that stocks produced using captive-spawning show losses of genetic diversity in progeny in cases where few broodstock and unequal sex ratios were used. Sole focus on production numbers is ill advised and may lead to irreversible losses of genetic diversity that may compromise the success of the supplementation program, and will likely cause reduction of N_e in the wild population. A recent theoretical study demonstrated that a breeding design that used factorial matings (every males breeds with every female) without equalization of progeny numbers maintained high levels of genetic diversity (high *Ne*) whilst maintaining production numbers (Fiumera et al. 2004). However in reality, this may not be possible in a small fish like the Rio Grande silvery minnow (limiting the number of eggs and sperm that individuals can produce). We plan to compare genetic outcomes of using paired matings and communal spawning in a collaborative effort with the Albuquerque Biopark in FY 2004 in an effort to determine the best breeding strategy for retaining genetic diversity.

3. It is imperative to develop and maintain a captive broodstock that reflects the levels of genetic diversity that are present in the wild population. The broodstock should be derived from hatchery reared wild-caught eggs where possible and not collection of adult wild fish. Collection of wild adult fish should be avoided to minimize the impact of propagation activities on the remaining adult population.

4. Findings reported in FY 2003 and presented here suggest that hatchery production should aim to repatriate sufficient numbers of adult fishes (i.e. capable of reproduction) to maintain 400,000 to 4 million fishes in the Middle Rio Grande (Turner et al. 2003). Propagation and supplementation should seek to repatriate as many fish as needed to achieve census numbers within this range (400,000 to 4 million potentially breeding adults) in the middle Rio Grande. Augmentation appears to have ameliorated losses of genetic diversity in wild stocks in 2002 and 2003 despite declines in adult density in the

wild. The decline of the wild population and increased reliance on augmentation with captively-reared Rio Grande silvery minnow, is likely to result in the continued erosion of genetic diversity regardless of supplementation efforts. Continued genetic monitoring is an essential to help evaluate the success of the propagation and augmentation program.

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Table 1: Sample information: sample origin (Wild by river reach, Wild-caught eggs- WcE, captive spawned- CS) (Dx- Dexter National Fish Hatchery and Technology Center, ABQ- Albuquerque Biological Park, MSB- Museum of Southwestern Biology), year collected, year of release, number of fish sampled for genetic analysis (N), release location. Numbers of broodstock used for captive spawning is given if available.

Origin	ID	Year Collected	Year Release	N	Fish released	Release location
Wild-Angostura	Wild 87	1987	NA	15	NA	NA
Wild-San Acacia	"	1987	NA	28	NA	NA
Wild-San Acacia	Wild 99	1999	NA	46	NA	NA
Wild-San Acacia	Wild 00	1999	NA	194	NA	NA
Wild- Isleta	Wild 01	2001	NA	64	NA	NA
Wild-San Acacia	"	2001	NA	64	NA	NA
Wild-Angostura	Wild 02	2002	NA	68	NA	NA
Wild-Isleta	"	2002	NA	109	NA	NA
Wild-San Acacia	"	2002	NA	200	NA	NA
Wild-Angostura	Wild 03	2003	NA	62	NA	NA
Wild-Isleta	"	2003	NA	62	NA	NA
Wild-San Acacia	"	2003	NA	34	NA	NA
Wild-Angostura	Wild 04	2004	NA	144	NA	NA
Wild-Isleta	"	2004	NA	12	NA	NA
Wild-San Acacia	"	2004	NA	6	NA	NA
WcE-SA-Dx	WcE-01	May 2001	Jan 2002	178	12,900	San Acacia
WcE-SA-ABQ	WcE-SA-01	May 2001	-	50	-	-
WcE-San Acacia	WcE-SA-02	May 2002	Dec 2002	81	41,500	Angostura
			Jan 2003	-	61,418	Angostura
			Jan 2004	-	48,000	Angostura
WcE-San Acacia-Dx	WcE-SA-03	May 2003	Apr 2004	51	32,950	Angostura
WcE-San Acacia-MSB	Eggs-03	May 2003	-	391	-	-
CS-San Acacia[3]	Not sampled	May 2000	2000	-	91,600	Angostura
CS-San Acacia[3]	Not sampled	May-Jun 2000	2000	-	112,000	Isleta
CS-ABQ	Cs-TFT-039	2001/02	Apr 2004	48	12,000	Angostura
Cs-Dx-01 (35 F and 136 M[1], 14 F and 14 M)[3]	Cs-Dx-04	2000/02	Apr 2004	50	17,250	Angostura
CS-ABQ	Cs-ABQ-01	2001	-	64	-	-
CS-Angostura (8 F and 6 M)	Cs-An-02	2002	-	51	-	
CS-San Acacia (15 F and 8 M)	Cs-SA-02	2002	-	53	-	-

[1] Female broodstock 2000 and 2002 wild-caught eggs collected at San Marcial. Male broodstock 2002 wild-caught eggs collected at San Marcial and F1 captive spawn from 2001 wild fish (*possibly only 2 fish produced these males*) (A-Mountain). [2] Paired matings (Dexter) between adults reared from wild-caught eggs collected in 2001 and wild caught adults collected 2002. [3] Released as larval fish.

Table 2: Summary statistics for 10 microsatellite loci for wild, hatchery reared wild-caught eggs (WcE) and captively spawned stocks (CS). Observed number of alleles per locus and sample, expected heterozygosity (H_E), observed heterozygosity, results (P- values) for tests for deviations from Hardy-Weinberg proportions (H1 = heterozygote deficiency) (HS- highly significant P < 0.0001).

Locus		Wild 1987	Wild 1999	Wild 2000	Wild 2001	Wild 2002	Wild 2003	Wild 2004	WcE DX-01	WcE SA-01	WcE SA-02	WcE AN-02	WcE SA-03	Eggs -03	CS-2001	CS AN-02	CS SA-02	CS-TFT 39	CS DX-04
Lco1	No Alleles	29	25	37	39	39	35	37	35	27	35	22	37	44	36	10	28	25	30
	H-W Test	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	0.81	0.047	HS	HS	0.970	HS	0.043	0.0004
	H_E	0.960	0.958	0.96	0.964	0.961	0.957	0.964	0.96	0.951	0.97	0.939	0.960	0.962	0.949	0.792	0.95	0.939	0.969
	H_O	0.829	0.846	0.731	0.897	0.823	0.874	0.835	0.576	0.760	0.72	0.955	0.961	0.757	0.821	0.86	0.857	0.958	0.83
Lco3	No Alleles	7	3	12	11	14	11	12	14	9	12	10	11	13	8	12	9	11	9
	H-W Test	0.350	0.410	0.490	HS	HS	0.095	0.103	HS	0.931	HS	HS	0.033	HS	0.892	0.006	0.478	HS	0.302
	H_E	0.729	0.787	0.752	0.703	0.777	0.791	0.815	0.791	0.747	0.84	0.81	0.767	0.766	0.752	0.6	0.744	0.772	0.754
	H_O	0.714	0.796	0.768	0.627	0.794	0.864	0.859	0.566	0.83	0.53	0.66	0.784	0.748	0.807	0.319	0.717	0.625	0.696
Lco4	No Alleles	8	3	8	10	15	9	12	13	7	11	6	6	16	5	5	5	6	4
	H-W Test	HS	0.454	0.011	HS	HS	0.003	HS	HS	HS	HS	HS	HS	HS	0.019	HS	HS	HS	0.072
	H_E	0.731	0.624	0.609	0.552	0.689	0.679	0.678	0.582	0.558	0.68	0.674	0.593	0.644	0.564	0.599	0.533	0.571	0.533
	H_O	0.395	0.537	0.428	0.333	0.59	0.846	0.402	0.44	0.28	0.27	0.22	0.176	0.576	0.24	0.26	0.447	0.274	0.422
Lco5	No Alleles	4	8	7	10	7	5	8	5	3	5	3	5	11	4	2	3	4	4
	H-W Test	0.032	0.027	0.007	HS	HS	1.000	HS	0.0008	0.941	0.0004	0.211	HS	HS	0.074	0.985	0.890	HS	0.088
	H_E	0.488	0.18	0.109	0.394	0.595	0.57	0.602	0.558	0.532	0.53	0.454	0.534	0.473	0.418	0.457	0.44	0.537	0.648
	H_O	0.5	0.45	0.456	0.339	0.442	0.772	0.456	0.671	0.638	0.59	0.4	0.588	0.467	0.457	0.571	0.5	0.592	0.587
Lco6	No Alleles	11	10	15	12	14	12	12	13	11	12	11	12	13	10	7	7	11	10
	H-W Test	0.037	0.009	0.085	0.145	HS	0.229	0.005	HS	0.0006	HS	0.207	0.005	0.002	0.642	HS	0.046	HS	0.001
	H_E	0.661	0.707	0.665	0.698	0.624	0.54	0.689	0.664	0.704	0.73	0.685	0.727	0.630	0.636	0.508	0.583	0.793	0.751
	H_O	0.59	0.610	0.627	0.669	0.464	0.485	0.689	0.463	0.542	0.43	0.708	0.633	0.603	0.661	0.412	0.423	0.643	0.522
Lco7	No Alleles	11	7	4	11	14	15	15	17	10	13	9	14	17	9	6	10	9	13
	H-W Test	0.008	0.106	HS	0.0005	HS	0.092	HS	HS	0.001	HS	HS	HS	HS	0.002	0.0003	HS	HS	HS
	H_E	0.859	0.779	0.835	0.796	0.805	0.785	0.796	0.858	0.786	0.87	0.851	0.85	0.860	0.809	0.662	0.821	0.803	0.85
	H_O	0.732	0.693	0.755	0.691	0.524	0.705	0.671	0.5	0.58	0.3	0.511	0.588	0.722	0.625	0.353	0.539	0.420	0.533
Lco8	No Alleles	12	11	17	16	21	20	21	20	12	12	13	15	-	14	10	14	12	17
	H-W Test	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	-	HS	HS	HS	HS	HS
	H_E	0.884	0.866	0.883	0.869	0.805	0.785	0.796	0.884	0.899	0.86	0.811	0.902	-	0.789	0.797	0.864	0.897	0.903
	H_O	0.585	0.500	0.601	0.496	0.612	0.564	0.774	0.425	0.542	0.44	0.48	0.647	-	0.625	0.28	0.481	0.479	0.689

		Wild 1987	Wild 1999	Wild 2000	Wild 2001	Wild 2002	Wild 2003	Wild 2004	WcE DX-01	WcE SA-01	WcE SA-02	WcE AN-02	WcE SA-03	Eggs-03	CS-2001	CS AN-02	CS SA-02	CS-TFT 39	CS DX-04
Ca1	No Alleles	4	3	8	8	11	7	14	12	4	6	2	7	-	4	2	5	5	8
	H-W Test	HS	HS	HS	HS	HS	0.006	HS	HS	0.0007	HS	HS -	0.0003	-	HS	0.060	0.035	0.008	0.0002
	H_E	0.285	0.231	0.632	0.610	0.329	0.093	0.671	0.532	0.57	0.37	0.02	0.621	-	0.55	0.076	0.274	0.43	0.803
	H_O	0.047	0.057	0.109	0.091	0.106	0.054	0.204	0.165	0.26	0.05	0.02	0.451	-	0.156	0.039	0.226	0.373	0.208
Ca6	No Alleles	7	8	8	9	18	10	12	17	10	11	9	9	9	10	6	9	8	10
	H-W Test	0.868	0.002	0.614	0.093	HS	0.972	HS	HS	0.075	HS	0.003	0.002	0.159	0.837	0.891	0.566	0.008	0.014
	H_E	0.593	0.744	0.654	0.706	0.785	0.778	0.806	0.827	0.851	0.75	0.738	0.785	0.651	0.684	0.798	0.718	0.674	0.803
	H_O	0.628	0.515	0.685	0.661	0.711	0.845	0.656	0.642	0.804	0.64	0.5	0.686	0.630	0.781	0.831	0.792	0.549	0.619
Ca8	No Alleles	23	8	31	27	31	28	28	27	21	25	24	23	-	20	12	21	22	19
	H-W Test	HS	HS	HS	HS	HS	HS	HS	HS	0.004	0.046	HS	0.004	-	HS	0.946	HS	0.127	0.006
	H_E	0.959	0.872	0.989	0.950	0.947	0.942	0.944	0.944	0.941	0.950	0.929	0.943	-	0.935	0.815	0.944	0.928	0.959
	H_O	0.625	0.235	0.714	0.805	0.686	0.673	0.862	0.536	0.900	0.520	0.750	0.823	-	0.491	0.902	0.423	0.882	0.787

Table 3: Summary statistics for microsatellite and mtDNA – ND4 loci for wild (1987, 1999-2004), hatchery reared wild-caught eggs, captively spawned Rio Grande silvery minnow. Sample size (*N*), expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness and average weighted inbreeding co-efficient (F_{IS}) are given over all loci. For ND4 the gene diversity (*h*) and nucleotide diversity and the observed number of haplotypes are given. * Eggs-03 summary data is based on seven microsatellite loci.

Origin	**Microsatellites**					**MtDNA- ND4**			
	N	H_E	H_O	Allelic Richness	F_{IS}	*N*	*h*	Nucleotide Diversity	N Haplotypes
Wild 1987	43	0.7132	0.5645	11.48	0.211	34	0.693	0.005	6
Wild 1999	46	0.6754	0.4969	9.000	0.268	44	0.427	0.004	5
Wild 2000	194	0.7307	0.5884	16.493	0.196	130	0.405	0.003	7
Wild 2001	128	0.7236	0.5609	11.993	0.226	99	0.648	0.004	8
Wild 2002	389	0.7387	0.5750	12.471	0.222	377	0.641	0.004	8
Wild 2003	169	0.7018	0.6664	11.401	0.051	168	0.529	0.004	9
Wild 2004	162	0.7855	0.6383	13.008	0.188	162	0.624	0.004	10
Eggs-03*	*348*	*0.7123*	*0.6433*	*11.65*	*0.098*	391	0.560	0.004	9
WcE-SA-01	50	0.7524	0.6136	10.887	0.186	51	0.648	0.004	6
WcE-Dx-01	178	0.7591	0.4984	12.628	0.340	157	0.624	0.004	8
WcE-AN-02	50	0.6893	0.5204	10.279	0.247	49	0.476	0.005	3
WcE-SA-02	81	0.7508	0.4432	12.219	0.410	81	0.703	0.006	8
WcE-SA-03	51	0.7668	0.6339	12.206	0.175	51	0.714	0.006	8
Cs-HA-01	64	0.7075	0.5666	10.098	0.200	58	0.460	0.002	5
Cs-An-02	51	0.6091	0.4840	6.760	0.207	51	0.000	0.000	1
Cs-SA-02	53	0.6877	0.5406	10.450	0.216	53	0.745	0.006	6
Cs-Dx-04	48	0.7751	0.5893	12.106	0.242	47	0.586	0.003	6
TFT-039	48	0. 7327	0. 5796	10.812	0.211	51	0.558	0.003	4

Table 4: Summary statistics for microsatellites (combined) screened from wild Rio Grande silvery minnow between 1987, 1999-2004 by river reach (An-Angostura, Is- Isleta, SA- San Acacia). Sample size (*N*), expected heterozygosity (H_E), observed heterozygosity (H_O), average weighted inbreeding co-efficient (F_{IS}), average number of alleles per locus and the number of mitochondrial ND4 haplotypes are shown.

Wild Population	*N*	H_E	H_O	F_{IS}	*Average alleles /locus*	*Mt-DNA Haplotypes*
1987-An	28	0.710	0.555	0.221	9.9	5
1987-SA	15	0.730	0.588	0.201	8.8	4
1999-SA	46	0.675	0.497	0.268	8.7	5
2000-SA	194	0.731	0.587	0.197	15.8	7
2001-Is	65	0.705	0.547	0.225	12.9	8
2001-SA	63	0.726	0.574	0.211	13.1	6
2002-An	67	0.769	0.610	0.209	13.3	6
2002-Is	121	0.749	0.579	0.228	15.5	6
2002-SA	201	0.711	0.561	0.211	16.8	7
2003-An	71	0.703	0.656	0.068	12.2	8
2003-Is	65	0.705	0.687	0.026	12.5	6
2003-SA	33	0.685	0.649	0.053	10.6	5
2004-An	141	0.783	0.638	0.186	16.4	9
2004-Is	15	0.777	0.652	0.165	9.3	5
2004-SA	6	0.736	0.600	0.200	5.7	3

Table 5: Mitochondrial ND4 haplotype frequencies among wild adults, eggs (Eggs-03), captively-spawned (CS) and reared (WcE) Rio Grande silvery minnows.

	A	C	D	E	F	I	J	K	M	N	O	P	Q	R	S
Wild 1987	0.500	0.118	0.235	0.059	0.029	-	-	-	0.059	-	-	-	-	-	-
Wild 1999	0.750	-	0.114	0.068	0.045	-	-	0.023	-	-	-	-	-	-	-
Wild 2000	0.762	0.015	0.136	0.136	0.115	0.008	-	0.007	-	-	-	-	-	-	-
Wild 2001	0.566	0.111	0.061	0.040	0.121	0.010	-	0.081	-	-	0.010	-	-	-	-
Wild 2002	0.541	0.204	0.149	0.011	0.058	-	0.003	0.032	-	-	0.003	-	-	-	-
Wild 2003	0.667	0.060	0.149	0.030	0.054	-	0.006	0.012	0.006	-	0.018	-	-	-	-
Wild 2004	0.593	0.086	0.105	0.025	0.074	0.012	-	0.049	0.019	-	0.031	0.006	-	-	-
WcE-DX-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-SA-02	0.481	0.222	0.049	0.012	0.136	-	-	0.049	-	-	-	0.012	-	-	-
WcE-AN-02	0.653	0.020	0.327	-	-	-	-	-	-	-	-	-		-	-
WcE-SA-03	0.490	0.078	0.196	0.059	0.098	-	-	0.039	0.020	-	0.020	-	-	-	-
Eggs-03	0.064	0.132	0.069	0.043	0.066	-	0.010	0.020	-	-	0.013	-	0.008	-	0.003
CS-2001	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-TFT-039	0.608	0.027	0.039	-	-	-	-	0.078	-	-	-	-		-	-
CS-DX-04	0.596	0.255	0.021	-	0.043	-		0.064	-	-	0.021	-		-	-

Table 6: Pairwise F_{ST} between populations (microsatellite data above the diagonal and Mt-DNA below the diagonal).

	Wild 1987	**Wild 1999**	**Wild 2000**	**Wild 2001**	**Wild 2002**	**Wild 2003**	**Wild 2004**	**WcE-Dx-01**	**WcE-SA-01**	**WcE-SA-02**	**WcE-An-02**	**WcE-SA-03**	**Eggs-03**	**Cs-2001**	**Cs-An-02**	**Cs-SA-02**	**TFT-039**	**Cs-Dx-04**
Wild 87	-	0.083	0.014	0.034	0.011	0.014	0.034	0.025	0.038	0.013	0.251	0.038	0.003	0.031	0.071	0.012	0.046	0.032
Wild 99	0.033	-	0.011	-0.014	0.034	0.070	0.000	0.028	0.016	0.057	0.099	0.016	0.019	0.012	0.151	0.063	0.103	0.050
Wild 00	0.141	0.014	-	0.009	0.029	0.029	0.021	0.020	0.028	0.019	0.045	0.028	0.006	0.013	0.084	0.018	0.059	0.027
Wild 01	0.071	0.003	0.010	-	0.033	0.054	0.021	0.028	0.032	0.037	0.040	0.032	0.010	0.017	0.113	0.031	0.074	0.042
Wild 02	0.023	0.019	0.063	0.021	-	0.006	0.021	0.017	0.025	0.010	0.019	0.025	0.015	0.029	0.073	0.014	0.061	0.021
Wild 03	0.022	-0.008	0.034	0.012	0.008	-	0.032	0.022	0.032	0.012	0.014	0.032	0.018	0.017	0.063	0.012	0.059	0.029
Wild 04	0.042	-0.005	0.021	-0.002	0.009	-0.001	-	0.006	0.008	0.013	0.031	0.008	0.027	0.010	0.081	0.040	0.055	0.016
WcE-Dx-01	0.041	0.008	0.036	0.004	0.004	0.007	0.001	-	0.026	0.007	0.027	0.006	0.024	0.011	0.071	0.018	0.045	0.012
WcE-SA-01	0.045	-0.005	0.016	-0.013	0.007	0.001	-0.009	-0.007	-	0.026	0.052	0.032	0.039	0.053	0.087	0.048	0.026	0.034
WcE-SA-02	0.083	0.028	0.029	-0.001	0.026	0.026	0.008	0.004	-0.005	-	0.014	0.019	0.017	0.015	0.075	0.013	0.038	0.019
WcE-An-02	-0.016	0.037	0.145	0.085	0.036	0.027	0.052	0.063	0.064	0.109	-	0.035	0.021	0.046	0.069	0.079	0.059	0.032
WcE-SA-03	0.017	0.011	0.090	0.036	0.010	0.006	0.018	0.023	0.018	0.051	-0.005	-	0.040	0.014	0.070	0.033	0.061	0.013
Eggs03	0.060	0.001	0.020	0.001	0.012	0.005	-0.001	-0.001	-0.007	0.007	0.073	0.034	-	0.014	0.066	0.008	0.056	0.027
CS2001	0.184	0.048	0.015	0.019	0.085	0.065	0.039	0.053	0.028	0.039	0.192	0.124	0.039	-	0.099	0.028	0.074	0.031
CSAN02	0.672	0.786	0.799	0.737	0.628	0.666	0.684	0.691	0.772	0.713	0.665	0.642	0.682	0.878	-	0.079	0.113	0.081
CSSA02	-0.008	0.041	0.132	0.072	0.045	0.040	0.057	0.058	0.049	0.087	0.010	-0.009	0.078	0.170	0.583	-	0.060	0.027
TFT-039	0.103	0.048	0.065	0.017	0.021	0.036	0.018	0.007	0.009	0.008	0.127	0.073	0.013	0.060	0.862	0.121	-	0.056
CS-DX-04	0.106	0.044	0.048	0.008	0.021	0.034	0.013	0.004	0.003	-0.004	0.130	0.071	0.008	0.047	0.867	0.115	-0.017	-

Table 7: a) Wild 2001 compared to WcE-Dx-01 (offspring) and Wild 2002 (recruits), b) Wild 2002 and WcE-Dx-01 (breeders) compared to WcE-SA-02 (progeny) and Wild 2003 (recruits), c) Wild 2003 and WcE-Sa-02 (breeders) compared to WcE-SA-03 (progeny) and Wild 2004 (recruits), d) Wild 2003 and WcE-SA-02 compared to Eggs-03 (progeny), WcE-SA-03 (progeny) and wild 2004 (recruits). This comparison is based on data from seven microsatellite loci (*Lco1*, *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7*, *Ca6*) as Eggs-03 were only screened for variation at these loci.

a)

Population	**Wild 2001**	**WcE-Dx-01**
Wild 2001	-	
WcE-Dx-01	0.0280	-
Wild 2002	0.0332	0.01679

b)

Population	**Wild 2002**	**WcE-Dx-01**	**WcE-SA-02**
Wild 2002	-		
WcE-Dx-01	0.0167	-	
WcE-SA-02	0.0102	0.0071	-
Wild 2003	0.0064	0.0219	0.0123

c)

Population	**Wild 2003**	**WcE-SA-02**	**WcE-SA-03**
Wild 2003	-		
WcE-SA-02	0.0123	-	
WcE-SA-03	0.0319	0.0189	-
Wild 2004	0.0317	0.0130	0.0077

d)

Population	**Wild 2003**	**WcE-SA-02**	**Eggs-03**	**WcE-SA-03**
Wild 2003	-			
WcE-SA-02	0.0128	-		
Eggs-03	0.0179	0.0178	-	
WcE-SA-03	0.0203	0.0237	0.0397	-
Wild 2004	0.0101	0.0094	0.0269	0.0096

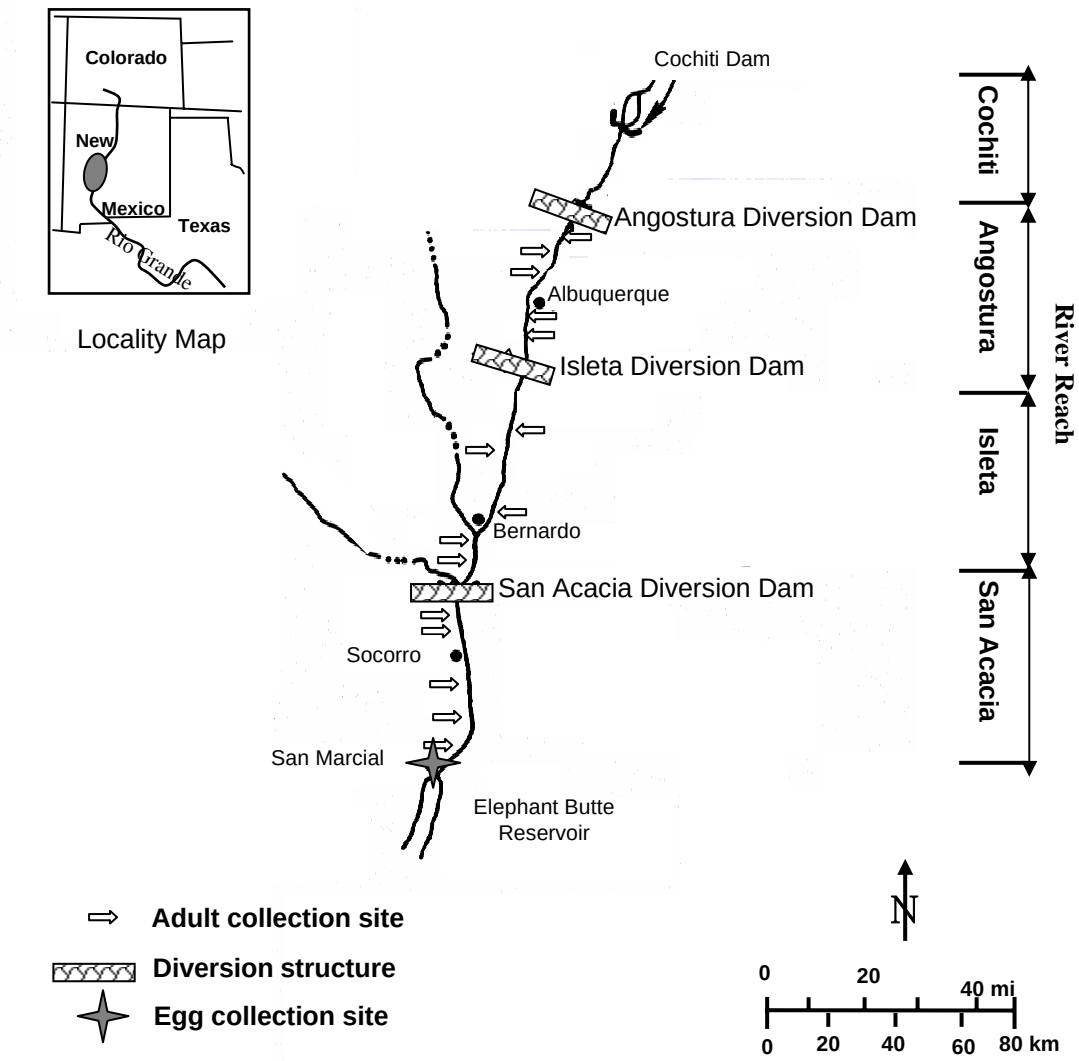

Figure 1: Map of Rio Grande silvery minnow current distribution (inset), adult (arrows) and egg collection (denoted by a star) sites in the middle Rio Grande, New Mexico. Four river reaches are shown which are delimited by three water diversion structures.

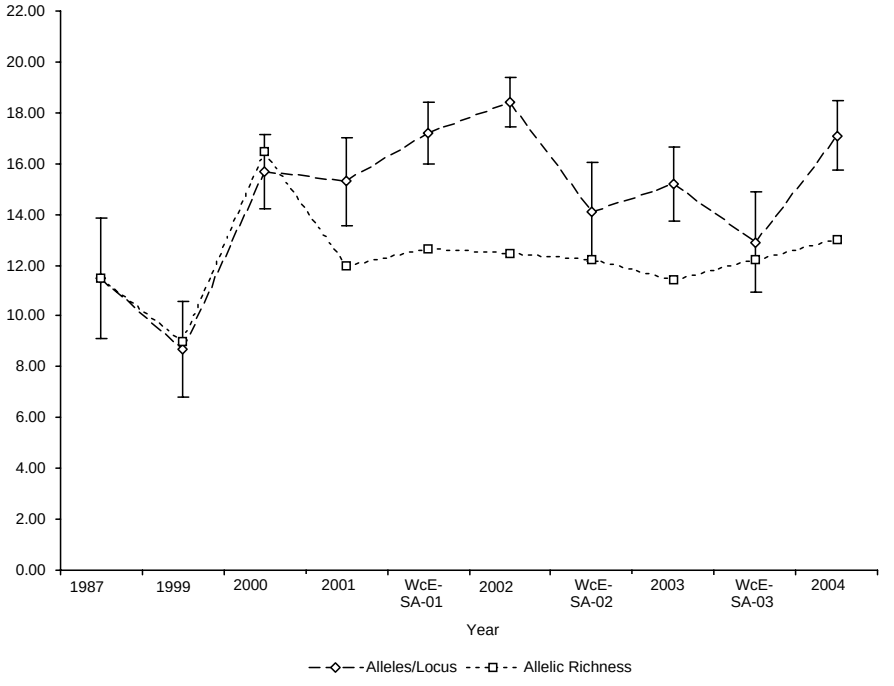

Figure 2: Allelic diversity (average numbers of alleles/locus and allelic richness) in wild population by year and in adult fish reared from wild-caught eggs. The reach and year that wild caught eggs were collected is shown (SA- San Acacia).

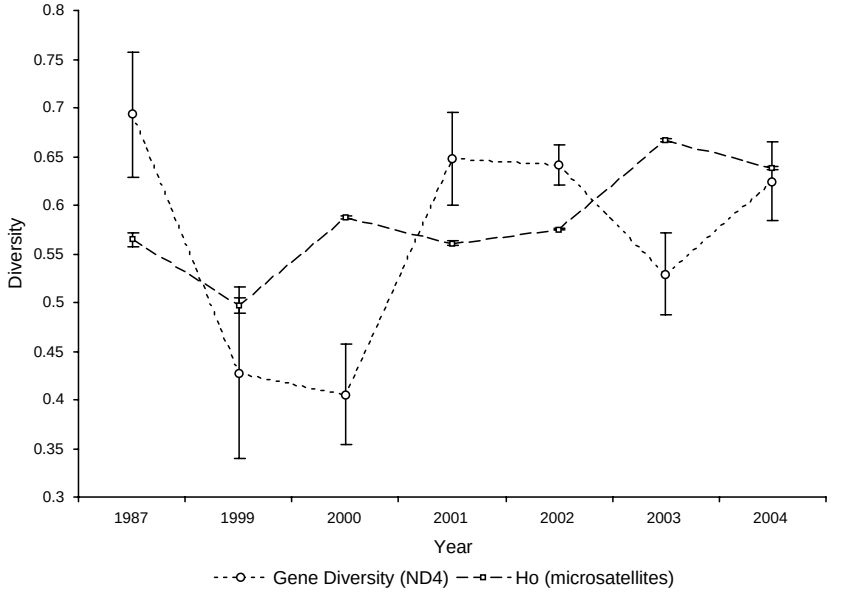

Figure 3: Gene diversity (ND4) and observed heterozygosity (microsatellites) is shown for wild fish collected in 1987 and between 1999 and 2004. 95% confidence intervals are given for genetic diversity statistics.

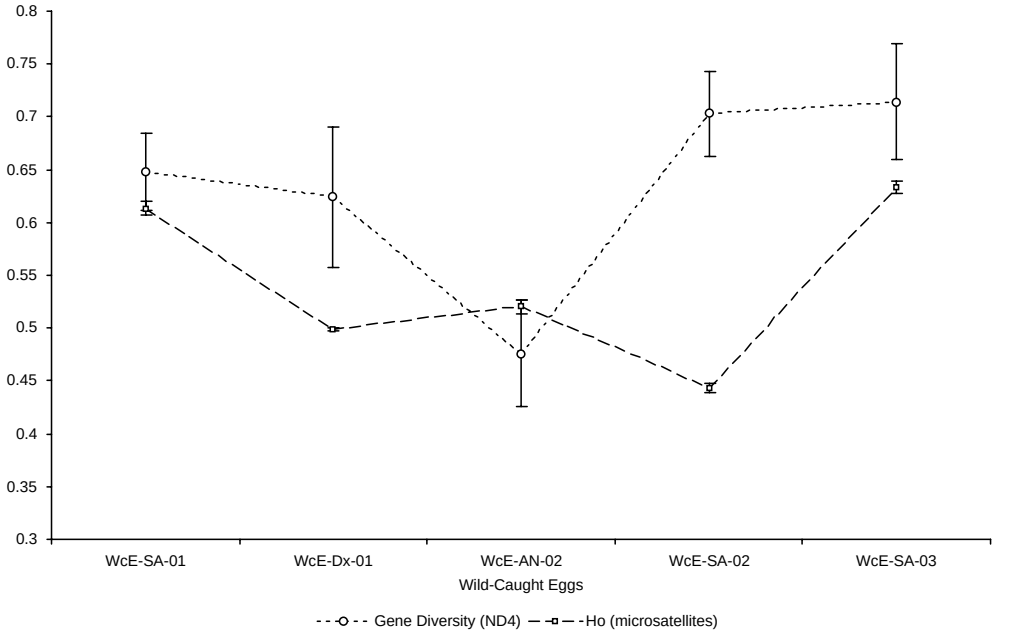

Figure 4: Gene diversity (ND4) and observed heterozygosity (microsatellites) is shown for adult fish reared from wild-caught eggs collected between 2001 and 2003. 95% confidence intervals are given for genetic diversity statistics.

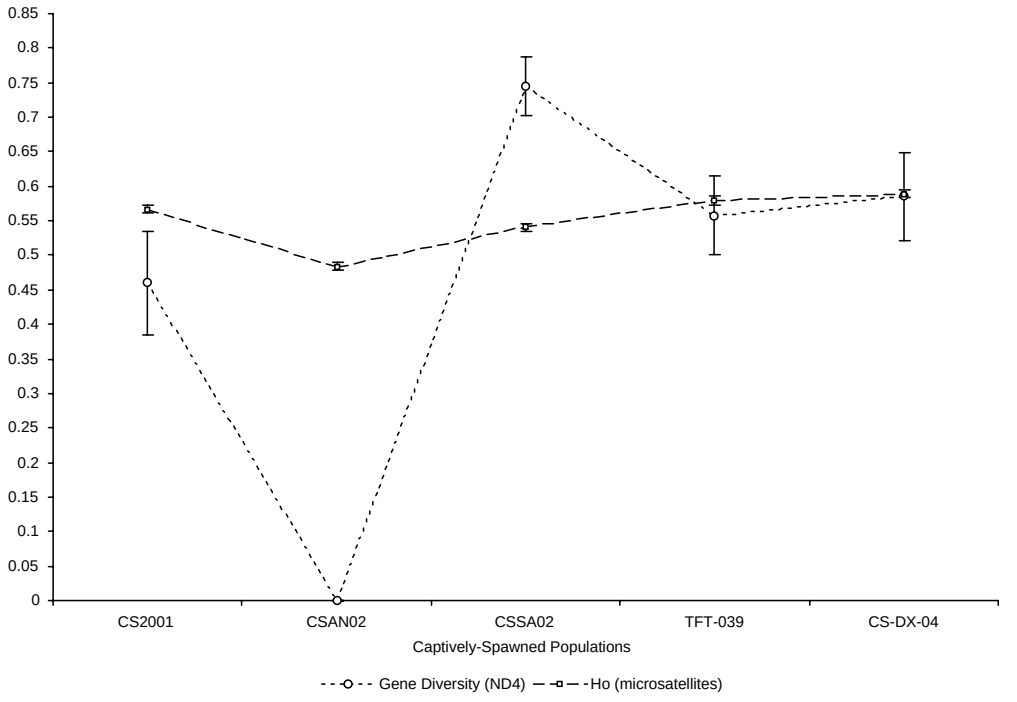

Figure 5: Gene diversity (ND4) and observed heterozygosity (microsatellites) is shown for Rio Grande silvery minnow progeny from captive spawning between 2001 and 2003. 95% confidence intervals are given for genetic diversity statistics.

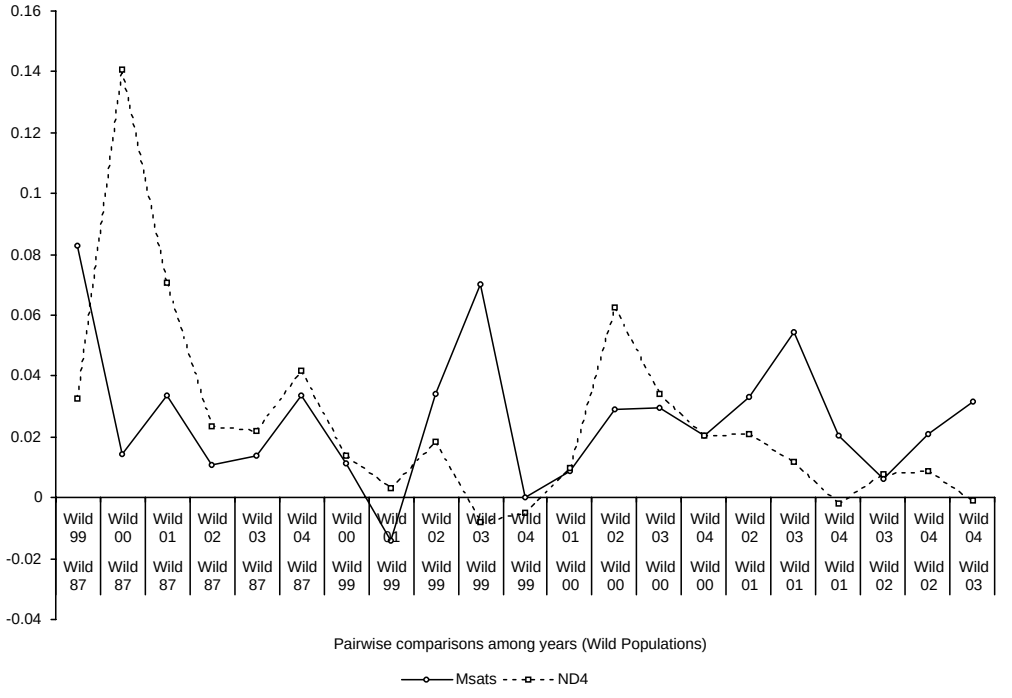

Figure 6: Pairwise F_{ST} values calculated from mitochondrial ND4 and microsatellite data among years for the wild Rio Grande silvery minnow population.

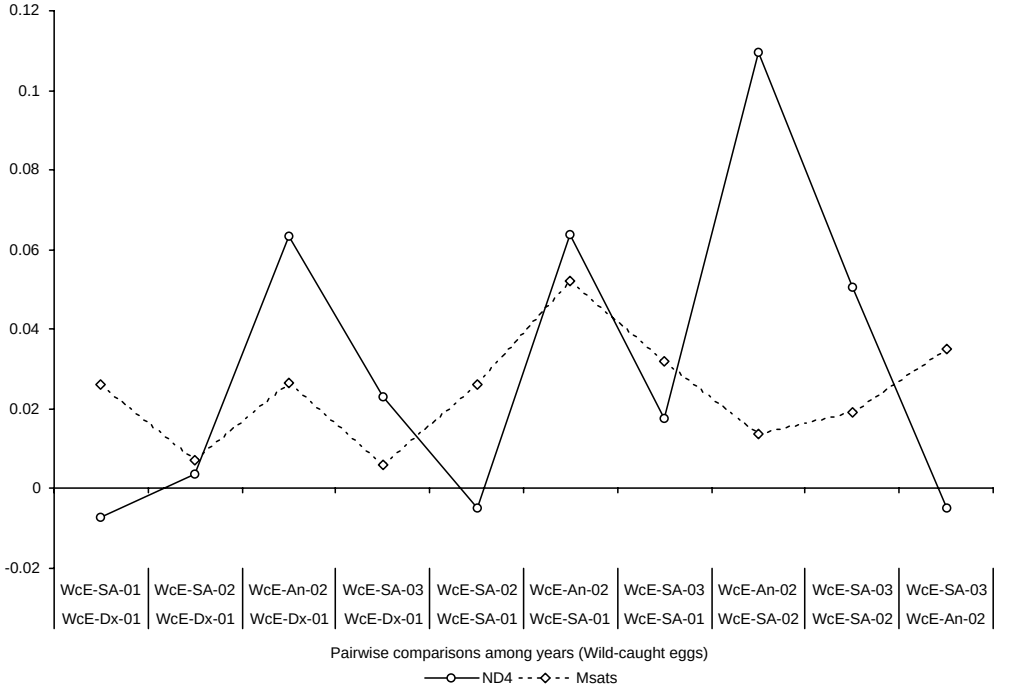

Figure 7: Pairwise F_{ST} values calculated from mitochondrial ND4 and microsatellite data among years for adult Rio Grande silvery minnows reared from wild-caught eggs.

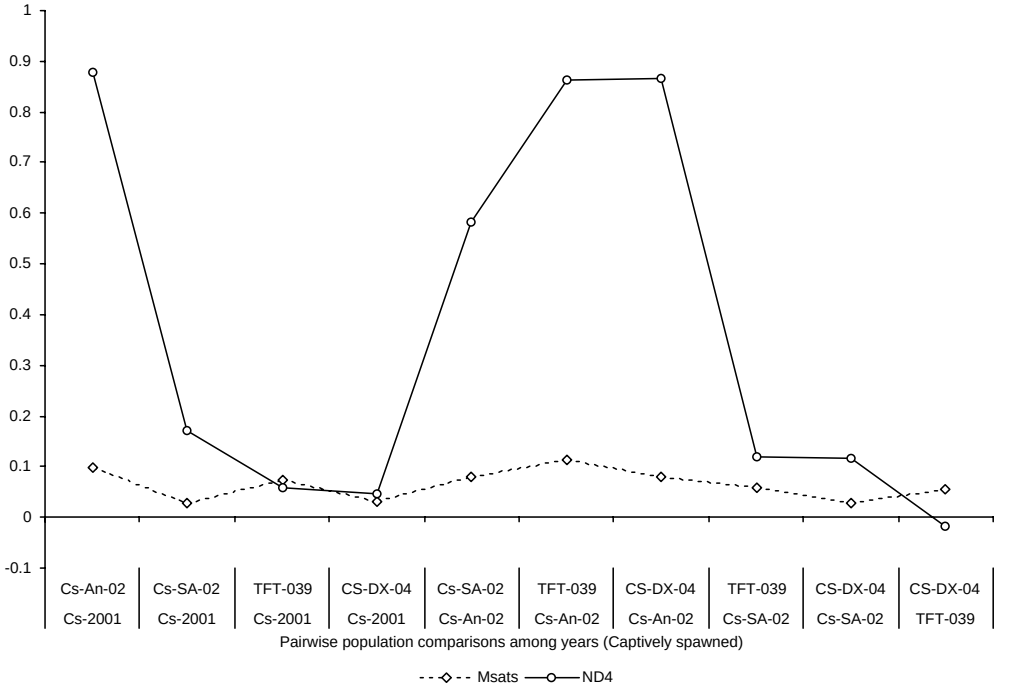

Figure 8: Pairwise F_{ST} values calculated from mitochondrial ND4 and microsatellite data among years for Rio Grande silvery minnow progeny from captive spawning.