

1     **Genetic effects of hatchery propagation and rearing in the endangered**  
2     **Rio Grande silvery minnow, *Hybognathus amarus*: a preliminary report.**

3

4

5           Megan J. Osborne, Melissa A. Benavides, Dominique Alò, and Thomas F. Turner.

6

7     Department of Biology and Museum of Southwestern Biology, University of New  
8     Mexico, Albuquerque New Mexico 87131.

9

10    Corresponding author: Dr. M. J. Osborne:

11    Telephone (505) 277 4191; FAX (505) 277 0304; email: mosborne@unm.edu

12

13

14

## Abstract

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17

The Rio Grande silvery minnow, *Hybognathus amarus*, is a federally endangered cyprinid now confined to the middle Rio Grande, New Mexico, in a fraction of its former range. The precipitous decline of the remaining wild population and lack of recruitment in the summer of 2000 prompted the collection and placement of eggs and wild fish in propagation facilities. The aim of this study was to assess the genetic effects of hatchery propagation in the Rio Grande silvery minnow using 10 microsatellite loci and partial mitochondrial ND4 sequences. Three hatchery stocks (2001, 2002 and 2003) and the wild source population (collected in 2001-2002) were considered. Principal findings were; (i) captively-spawned and reared Rio Grande silvery minnow had depleted levels of allelic diversity but similar levels of heterozygosity to the wild population, and (ii) fish raised from wild-caught eggs maintained similar levels of allelic diversity but had higher inbreeding coefficients than the wild source stock. With the repatriation of over 500,000 Rio Grande silvery minnow to the Rio Grande the genetic effects of propagation are likely to impact the remaining wild population, especially as numbers in the wild continue to decline.

1 **Introduction**

2           The Rio Grande silvery minnow (*Hybognathus amarus*) was previously one of the  
3 most widespread and abundant fish species in the Rio Grande basin with a distribution  
4 extending from northern New Mexico in the Rio Grande and Pecos River to the Gulf of  
5 Mexico (Pflieger 1980). *Hybognathus amarus* is now confined to approximately 5% of its  
6 former range in a 280-kilometer stretch of the middle Rio Grande from Cochiti Dam to  
7 the head of Elephant Butte Reservoir (Figure 1). Drastic range reduction and steady  
8 decline of the remaining population led the species to be listed as endangered (Federal  
9 Register 1994). Factors responsible for decimation of wild populations of *H. amarus*  
10 include habitat degradation, fragmentation of the Rio Grande by diversion structures and  
11 dams, river drying and intermittency, introduction of non-native species and flow  
12 alterations (Bestgen and Platania 1991).

13           Precipitous decline of adult numbers and poor recruitment of *H. amarus* in the  
14 summer of 2000 led managers to collect eggs and adult fishes from the wild and place  
15 them in propagation facilities (Davenport and Brooks 2003). Captive-rearing enhances  
16 survival of early life stages by reducing mortality imposed by predation, resource  
17 limitation, and catastrophic events. The primary goal of supportive breeding is to  
18 increase the reproductive output of the captive segment of the population and in doing so,  
19 to boost the wild adult census population size (Palm et al. 2003). Propagation efforts  
20 should also aim to provide fish that contribute to the long-term viability of the wild  
21 population, and so should strive to maintain the species' genetic diversity.

22           Although adult census size of the wild population may be increased by supportive  
23 breeding and subsequent introductions, there are risks associated with such measures.  
24 Detrimental genetic impacts include introduction of non-adaptive traits (Lynch *et al.*  
25 1995, Heath et al. 2003), reduction in the effective population size (Ryman and Laike

1 1991), inbreeding depression (Frankham 1995) and maladaptive behavioral changes  
2 (Hindar et al. 1991). Hatchery-reared fish may be depauperate of overall genetic  
3 diversity and this deficiency may ultimately reduce variability in wild recipient  
4 populations (Tringali and Bert 1998). This will be most evident if few founders are used  
5 as brood-stock. For a hatchery population to retain 99% of the heterozygosity of the wild  
6 population 50-500 effective founding breeders has been recommended (Ryman and Stahl  
7 1980, Frankel and Soulé 1981, Frankham 1995). A small brood-stock is expected to lose  
8 heterozygosity and exhibit lowered viability and fecundity as a result of inbreeding  
9 depression (Falconer 1981, Ralls and Ballou 1983).

10 Conservation and management plans for threatened and endangered fishes often  
11 place heavy emphasis on captive propagation and supportive breeding as primary tools  
12 for species recovery (Hedrick *et al.* 2000). It is imperative therefore that the genetic  
13 effects (in the hatchery fish and in the wild recipient population) of such measures be  
14 considered and understood. To date, the majority of studies have focused on species  
15 important to the fisheries industry, usually members of family Salmonidae (Hindar *et al.*  
16 1991; Wang *et al.* 2002). Salmonids have very different life histories compared to warm-  
17 water species like *H. amarus* (family Cyprinidae), and so, it may be inappropriate to base  
18 management practices solely on these studies. Of the 114 threatened and endangered  
19 fishes in North America, over a third are cyprinids. At least five cyprinid species are  
20 being captively-propagated in recovery efforts (US FWS 2003). Between 2000 and 2003  
21 over 500,000 hatchery-reared and propagated *H. amarus* were released in the middle Rio  
22 Grande (Remshardt 2002, Davenport and Brooks 2003). The aim of the present study is  
23 to evaluate the genetic effects of hatchery propagation in *H. amarus*.

24

## 1 **Materials and Methods**

### 2 *Sampling Localities and Methods*

3 Wild *Hybognathus amarus* were sampled from the middle Rio Grande, New  
4 Mexico. Three water-diversion structures (from north to south- Angostura Diversion  
5 Dam, Isleta Diversion Dam, and San Acacia Diversion Dam) divide the middle Rio  
6 Grande into four reaches: (i) Cochiti [36 kms] (ii) Angostura [65 kms] (iii) Isleta [86  
7 kms] and (iv) San Acacia [92 kms]. The present study focuses on the latter three reaches  
8 because *H. amarus* are now extremely rare in the Cochiti reach (Bestgen and Platania  
9 1991). Wild adult *H. amarus* were collected prior to spawning (December 2001 through  
10 March 2002) by seining and backpack electrofishing. Captured fishes were anesthetized  
11 in MS-222 (Tricaine Methane Sulfonate 200mg/L river water) at the capture site and a  
12 small piece of caudal fin was removed from each individual ( $n=389$ ). Fishes were  
13 allowed to recover in untreated river water prior to release.

14 Three year classes of hatchery reared and/or propagated *H. amarus* were  
15 considered. Year 2001 fishes (referred to hereafter as Hatchery 2001) were raised from  
16 captively-spawned wild caught adults (collected from the San Acacia reach in 2000). It is  
17 unknown how many broodstock were used in captive spawning. Collection of eggs for  
18 propagation activities were made during the peak spawning period that occurred from 8<sup>th</sup>  
19 – 11<sup>th</sup> May 2001 (Hatchery 2002 sample) and 17<sup>th</sup> – 19<sup>th</sup> May 2002 (Hatchery 2003  
20 sample). Approximately 100,000 and 922,000 eggs were collected in 2001 and 2002  
21 respectively. Collections of drifting eggs occurred 16 kilometers downstream of the San  
22 Marcial railroad bridge (Socorro County) in the San Acacia reach of the Rio Grande using  
23 modified Moore Egg collectors (Altenbach et al. 2000). Eggs were raised in propagation  
24 facilities and fin clips were taken from these fish (Hatchery 2002 and Hatchery 2003  
25 samples) prior to their release and stored in 95% ethanol. DNA was extracted from air-

1 dried fin clips using standard proteinase-k digestion and organic extraction methods  
2 (Hillis et al. 1996).

3

#### 4 *Characterization of genetic diversity: Microsatellites*

5       Individuals were screened for genetic variation at ten microsatellite loci: *Lco1*,  
6 *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7*, and *Lco8* (Turner et al. 2004) and *CA1*, *CA6*, and *CA8*  
7 (Dimsoski et al. 2000). Microsatellite loci were visualized using fluorescently labeled  
8 forward primers. The following microsatellites were amplified (in a 10µL reaction  
9 volume) using multiplex PCR: *Lco3*, *Lco4*, and *Lco5* (1X PCR buffer, 2mM MgCl<sub>2</sub>,  
10 125µM dNTPs, 0.40µM each primer, 0.375 units TAQ polymerase); *Lco6* and *Lco7* (1X  
11 PCR buffer, 2.5mM MgCl<sub>2</sub>, 125µM dNTPs, 0.40µM each primer, 0.375 units TAQ  
12 polymerase); *CA1* and *CA6* (1X PCR buffer, 2mM MgCl<sub>2</sub>, 125µM dNTPs, 0.40µM each  
13 primer, 0.375 units TAQ polymerase). The remaining microsatellites were amplified  
14 alone (*Lco1*, *Lco8*, and *CA8*) (1X PCR buffer, 2.5mM MgCl<sub>2</sub>, 125µM dNTPs, 0.50µM  
15 each primer, 0.375 units TAQ polymerase). PCR cycling conditions were: one  
16 denaturation cycle of 94°C for 2 mins followed by 30 cycles of 94 °C for 20s, 48°C  
17 (*Lco6*, *Lco7*, *CA1*, *CA6*) or 50°C (*Lco3*, *Lco4*, *Lco5*, *Lco8*) or 52°C (*Lco1*, *CA8*) for 20  
18 sec, 72°C for 30s. Prior to electrophoresis 1.2µL of PCR product was mixed with 1.2µL  
19 of a solution containing 62.5% formamide, 25% bromophenol blue, 12.5% Genescan  
20 ROX350 (ABI PRISM, Applied Biosystems) size standard and denatured at 94°C for 2  
21 min and placed on ice. Products were electrophoresed in an ABI377 Prism (Applied  
22 Biosystems) automated sequencer and analyzed with GeneScan Version 3.1.2 (Applied  
23 Biosystems) software.

24

25 *MtDNA-ND4*

1           A 295 base-pair fragment of the mitochondrial ND4 gene was amplified (10  $\mu$ L  
2 reaction) using the following conditions: 1  $\mu$ L DNA (50-100 ng/ $\mu$ L), 1X reaction buffer,  
3 2.5mM MgCl<sub>2</sub>, 125 $\mu$ M dNTPs, 0.50 $\mu$ M forward (5'- GAC CGT CTG CAA AAC CTT  
4 AA - 3') and reverse primer (5'- GGG GAT GAG AGT GGC TTC AA -3'), 0.375 units  
5 TAQ polymerase. PCR parameters were initial denaturation of 94°C for 2 mins followed  
6 by 30 cycles of 94°C for 30s, 52°C for 30s and 72°C for 30s. Single stranded  
7 conformational polymorphism (SSCP- Sunnucks et al. 2000) was used to characterize the  
8 genetic diversity in *H. amarus*. To confirm haplotype designations a proportion of  
9 variants were sequenced from each gel using ABI BigDye Terminator cycle sequencing  
10 kit and an ABI377 Prism automated sequencer. Sequencher Version 4.1.2 (Gene Codes)  
11 software was used to read sequences.

12

### 13 *Data Analysis*

14           Microsatellite data were analyzed using GENEPOP Version 3.1d (Raymond and  
15 Rousset 1995) and FSTAT Version 2.9.3.2 (Goudet 1993). For each population and locus  
16 gene diversity, number of alleles, allelic richness (based on the minimum sample size of  
17 54) and  $F_{IS}$  were calculated using FSTAT. Nei's estimation (Nei 1987) of heterozygosity  
18 was obtained for each locus and over all loci. Each locus and population was tested for  
19 deviations from Hardy-Weinberg expectations. Global tests for linkage disequilibrium  
20 were performed for all pairs of loci (Markov chain parameters were dememorization  
21 5000, batches 500 and iterations per batch 5000).

22

### 23 *Analyses of Population Structure*

24           For microsatellites and mtDNA-ND4 hierarchical analysis of molecular variance  
25 (AMOVA) was used to partition standardized genetic variance into differences among

1 groups (two groups- Hatchery stocks and Wild population) ( $F_{CT}$ ); differences between  
2 populations within groups ( $F_{SC}$ ); and among all populations ( $F_{ST}$ ). Weir and Cockerham's  
3 (1984)  $F$ -statistics were obtained using AMOVA as implemented in Arlequin (Schneider  
4 *et al.* 2000).  
5

## 1 **Results**

### 2 *Genetic diversity- Microsatellites*

3           There were between eight (*Lco5*) and 51 (*Lco1*) alleles in the 10 loci considered  
4 (Table 1). Allelic richness ranged from 3.844 (*CA1*) to 32.763 (*Lco1*) (Table 1). With  
5 the exception of *Lco8*, allelic richness was lowest at all loci for the Hatchery 2001  
6 population. Significant linkage disequilibrium was identified between a single pair of  
7 loci *Lco6* and *Lco7* ( $P < 0.001$ ). In the Hatchery 2001 population, allele frequencies at  
8 *Lco3*, *Lco5*, *Lco6* and *CA6* did not differ significantly from Hardy-Weinberg expectation  
9 (Table 1). The remaining loci in the Hatchery 2001 population and all loci in the  
10 remaining populations (San Acacia, Isleta, Angostura, Hatchery 2002, and Hatchery  
11 2003) deviated from Hardy-Weinberg expectations ( $P < 0.01$ ). A Global test revealed a  
12 deficiency of heterozygotes for all populations and loci ( $P < 0.0001$ ). Over all loci,  $F_{IS}$   
13 values ranged from 0.200 (Hatchery 2001) to 0.416 (Hatchery 2003) (Table 2).  $F_{IS}$  for  
14 the Hatchery 2003 stock was twice that in the wild population (0.222) (Table 2).

15

### 16 *Mitochondrial DNA- ND4*

17           Eleven ND4 haplotypes were detected among 670 individuals. The wild,  
18 Hatchery 2002, and Hatchery 2003 populations each had eight haplotypes, whereas the  
19 captively spawned Hatchery 2001 population had five haplotypes. The haplotypes  
20 differed by one to nine transitions, with sequence divergence (Kimura two-parameter  
21 method) (Kimura 1981) ranging from 0.34 % to 2.43 %. In all populations haplotype A  
22 was the most common (Table 3). Four haplotypes were present as singletons (J, N, O, P).

23

### 24 *Population Structure-Microsatellites*

25           Standardized genetic variance attributable to differences among river reaches was

1  $F_{ST} = 0.0138$  ( $P < 0.001$ ) and among the three hatchery populations was  $F_{ST} = 0.0153$  ( $P <$   
2  $0.001$ ). A significant proportion of genetic variation was explained by differences  
3 between wild and hatchery populations ( $F_{CT} = 0.009$ ,  $P < 0.001$ ).

4

5 *Mt-DNA ND4*

6         Significant genetic variation was attributable to differences among populations  
7 within groups (Hatchery and Wild) ( $F_{SC} = 0.010$ ,  $P = 0.003$ ). No significant variation  
8 was attributable to differences between hatchery and wild populations ( $F_{CT} = 0.006$ ,  $P =$   
9  $0.100$ ). Significant variation was explained by differences among the three hatchery  
10 stocks (2001, 2002 and 2003) ( $F_{ST} = 0.026$ ,  $P = 0.004$ ). No significant genetic variation  
11 was explained by differences among the three river reaches ( $F_{ST} = 0.003$ ,  $p = 0.644$ ).

12

1 **Discussion**

2 Captive propagation should aim to maintain genetic diversity to ensure long-term  
3 viability of the wild population. Conservation of genetic diversity in a population  
4 requires that the composition (allelic diversity and heterozygosity) and distribution  
5 (spatial distribution and heterogeneity) of the variation is preserved (Brown *et al.* 2000).  
6 Several findings presented here suggest that the goal of retaining genetic variability in the  
7 captively-propagated *H. amarus* is not being realized.

8  
9 *Comparison of Wild and Captively-propagated stocks (Hatchery 2001)*

10  
11 Observed heterozygosity of the captively-spawned population (Hatchery 2001) is  
12 equivalent to that seen in the wild population, but allelic diversity is much lower. The  
13 loss of alleles and haplotypes from captively-spawned *H. amarus* is not surprising as most  
14 rare alleles will not be sampled when the brood-stock is founded by relatively few  
15 individuals. If rare alleles are sampled they are likely to be lost rapidly by genetic drift  
16 (Lacy 1987) as the probability of retention is directly proportional to the effective  
17 population size,  $N_e$  (Allendorf 1986). For the captive brood-stock obtained in year 2000  
18 (Hatchery 2001),  $N_e$  is roughly equal to the number of breeders that actually contributed  
19 offspring to the subsequent generation. Lowered allelic diversity in captively-spawned  
20 stocks is consistent with the observation that rare alleles are lost more rapidly than  
21 heterozygosity when  $N_e$  is reduced substantially (as in a ‘genetic bottleneck’ event) (Lacy  
22 1987), which implies that  $N_e$  was smaller in the captive brood-stock than in the wild  
23 source population.

24 The detection of very few, closely related mitochondrial ND4 haplotypes in the  
25 670 *H. amarus* screened is consistent with a population that has experienced bottleneck  
26 events in the recent past (Avise 2000). During bottleneck events the probability of  
27 retaining an allele is directly proportional to its frequency in the population (Allendorf

1 1986) hence rare alleles are more likely to be lost during such events. If severe  
2 population reductions occur in the northern reaches of the Rio Grande they can only be  
3 re-colonized by artificial translocations of individuals as diversion structures prevent  
4 upstream movement.

5

#### 6 *Hatchery-reared progeny of wild-caught eggs*

7 *Hybognathus amarus* releases pelagic eggs that drift substantial distances (up to  
8 72 km/day prior to hatching) with river currents (Platania and Altenbach 1998). It is  
9 predicted that drifting eggs collected from the lowest reach (San Acacia) will represent  
10 the genetic diversity seen over the entire population (based on data found in Platania and  
11 Dudley 2000). Samples from the three reaches of the middle Rio Grande were pooled for  
12 comparisons with the hatchery stocks to test this prediction.

13 At several loci, allelic richness in hatchery (2002, 2003) stocks actually exceeded  
14 that observed in the wild population. The pelagic nature of *H. amarus* eggs means that a  
15 substantial proportion of eggs are likely to be transported to unsuitable nursery habitats  
16 where they are subject to heavy mortality. It is expected that only a small fraction of the  
17 total spawn will be successfully recruited into the adult population (Alò and Turner, in  
18 review). Survival of eggs and subsequent early life stages is greater in propagation  
19 facilities than in the wild population. Increased allelic richness in hatchery stocks (2002,  
20 2003) might be explained by reduction of variance in mortality of the hatchery stocks.

21

#### 22 *Implications for species recovery*

23 *Hybognathus amarus* is a short-lived fish with few individuals surviving beyond  
24 13 months in the wild (Propst 1999). The impact of supplementation is likely to be more  
25 extreme and rapid in *H. amarus* than in long-lived species because repatriated hatchery-

1 reared fish can contribute immediately to the reproductive output of the recipient wild  
2 population. Theory indicates that when captively-reared fish represent a large proportion  
3 of the total number of breeders in the wild population, effective population size is reduced  
4 in subsequent generations (Ryman and Laikre 1991). This will be exacerbated if the  
5 reproductive contribution among broodstock individuals is unequal due to factors such as  
6 incomplete mixing of sperm and eggs, sperm competition, variation in female fecundity  
7 and differential survival of matings (Brown *et al.* 2000). This would result in a lower  
8 than predicted  $N_e$  in the captive population. Selecting broodstock from sampling  
9 localities throughout the species distribution, maximizing the number of broodstock used,  
10 and equalizing the reproductive contributions of individuals could help to reduce, but not  
11 eliminate, loss of genetic variation in captively-spawned fish.

12 Hatchery stocks from wild caught eggs in 2002 and 2003 retain only about 78% of  
13 the heterozygosity of the parental source population. This trend in the captively-  
14 propagated *H. amarus* is a concern given that from the 2003 captive stock alone, 130 000  
15 fish have been repatriated to the Rio Grande. If these fish spawn successfully, then  
16 theory predicts increasingly high  $F_{IS}$  values will be apparent in subsequent generations.  
17 Genetic effective size of the wild population of *H. amarus* is already very small ( $N_e \approx 70$ )  
18 (Alo and Turner, *in review*), and problems associated with small effective size of the wild  
19 population may be compounded by supplementation. For example, loss of heterozygosity  
20 and allelic diversity in captive or refugial populations has been reported in a large number  
21 of fish species including the mosquito fish (*Gambusia affinis*) (Stockwell *et al.* 1996),  
22 cutthroat trout (*Onchorhynchus clarki*) (Allendorf and Phelps 1980) and Atlantic salmon  
23 (*Salmo salar*) (Cross and King 1983). There are also numerous examples where gene  
24 frequencies have shifted in the wild population to resemble those of the hatchery stocks  
25 (Altukov 1981). Shifts in allele frequencies (towards those seen in the hatchery stocks)

1 can be expected in the wild population of *H. amarus* especially with continued decline in  
2 number of wild fish continued release of hatchery fish.

3         Although the present study has only considered neutral genetic markers, hatchery  
4 propagation can affect genes that are under selection by either relaxation of selection  
5 pressures found in the wild (such as those imposed by predation, egg and larval transport,  
6 etc.) or by the imposition of domestication selection. If natural selection is relaxed, traits  
7 can be promoted that are advantageous in the captive environment, but are maladaptive in  
8 the natural habitat (e.g. Heath et al. 2003). Alteration of selective regimes can lead to  
9 detrimental genetic changes to hatchery stocks that can be transferred to the wild  
10 population by augmentation. For example, relaxation of selection can lead to  
11 proliferation of deleterious alleles in subsequent generations of captive fish (Lynch and  
12 O’Hely 2001). If large numbers of hatchery-reared fish are introduced, the wild  
13 population will be swamped with potentially less fit hatchery-raised individuals.

14         Although introduction of hatchery-raised fish may temporarily increase adult  
15 census population size, the status of the wild population of *H. amarus* is unlikely to be  
16 improved unless the primary reasons for the populations continued decline are addressed.  
17 Our data indicate that captive propagation, hatchery rearing, and supplementation of wild  
18 populations is likely to lead to genetic changes that will decrease the probability of long-  
19 term persistence of *H. amarus* in the wild. Our recommendation is that propagation and  
20 supplementation be used sparingly as a tool to prevent extinction from catastrophic  
21 events, but not as a panacea for the long-term conservation of *H. amarus*.

1 **Acknowledgements**

2

3 Our sincere thanks are extended to S. P. Platania (SPP), R. K. Dudley, W. H.  
4 Brandenburg, A. M. Snyder, M. A. Farrington, C. S. Altenbach, M. D. Porter, J. E.  
5 Brooks, D. L. Propst, M. Ulibarri, the Albuquerque Fishery Resources Office of the US  
6 Fish and Wildlife Service, and the Museum of Southwestern Biology for technical and  
7 logistic support throughout the project. Funding was provided by New Mexico  
8 Department of Game and Fish, US Forest Service, US Bureau of Reclamation, US Fish  
9 and Wildlife Service and the National Science Foundation. G. Moyer provided editorial  
10 assistance. Rio Grande silvery minnow were collected under Federal Fish and Wildlife  
11 Permits TE001623-0 (SPP) and TE038055-0 (TFT) and New Mexico Department of  
12 Game and Fish Scientific Collecting Permits 1896 (SPP) and 3015 (TFT).

13

14

1 **References**

2

3 Allendorf, F. 1986. Genetic drift and the loss of alleles versus heterozygosity. *Zoo*  
4 *Biology* 5: 181-190.

5 Allendorf, F., and S. Phelps. 1980. Loss of genetic variability in a hatchery stock of  
6 cutthroat trout. *Transactions of the American Fisheries Society* 109: 537-543.

7 Alo, D. and T. Turner. Effects of river fragmentation on genetic diversity of the Rio  
8 Grande silvery minnow. *In review*.

9 Altukhov, Y. 1981. The stock concept from the view point of population genetics.  
10 *Canadian Journal of Fisheries and Aquatic Sciences* 38: 1523-1538.

11 Avise, J. 2000. *Phylogeography: the history and formation of species*. Harvard  
12 University Press. Cambridge, Massachusetts.

13 Bestgen, K., and S. Platania. 1991. Status and conservation of the Rio Grande silvery  
14 minnow, *Hybognathus amarus*. *Southwestern Naturalist* 36: 225-232.

15 Brown, B., T. Gunter, J. Waters, and J. Epifanio. 2000. Evaluating genetic diversity  
16 associated with propagation-assisted restoration of American shad. *Conservation*  
17 *Biology* 14 (1): 294-303.

18 Cross, T., and J. King. 1983. Genetic effects of hatchery rearing in Atlantic salmon.  
19 *Aquaculture* 33: 33-40.

20 Davenport, S. and J. Brooks. 2003. Propagation of the Rio Grande silvery minnow fiscal  
21 year 2001 year end report. U.S. Fish and Wildlife Service, Fishery Resources  
22 Office, Albuquerque, New Mexico.

23 Dimsoski, P., G. Toth, and M. Bagley. 2000. Microsatellite characterization in central  
24 stoneroller *Camptostoma anomalum* (Pisces : Cyprinidae). *Molecular Ecology* 9:  
25 2187-2189.

26 Falconer, D. 1981. *Introduction to Quantitative Genetics*. 2<sup>nd</sup> Edition. Longman, London.

27 Frankel, O., and M. Soule. 1981. *Conservation and Evolution*. Cambridge University  
28 Press, Cambridge, United Kingdom.

29 Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a  
30 review. *Genetical Research* 66: 95-107.

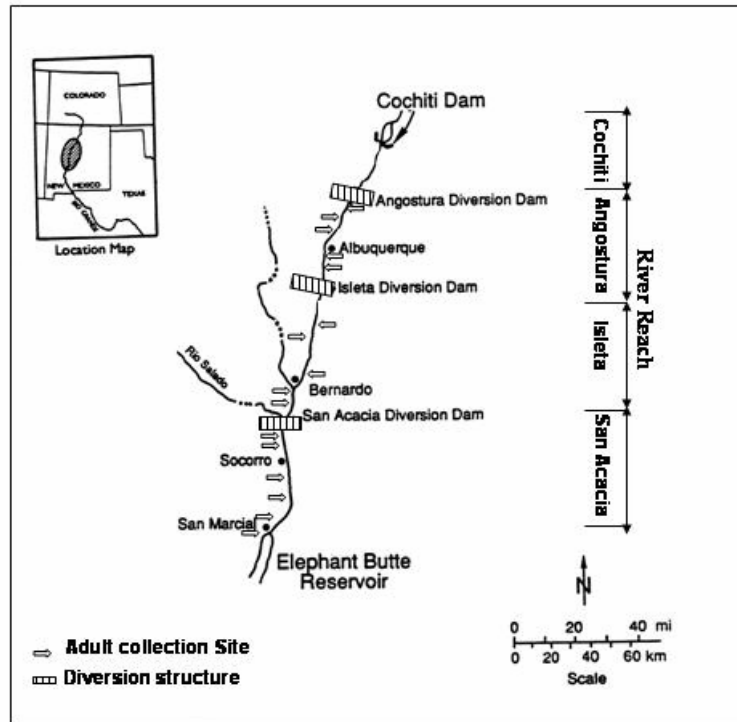
31 Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics.  
32 *Journal of Heredity* 86: 485-486.

33 Heath, D., J. Heath, C. Bryden, R. Johnson, and C. Fox. 2003. Rapid evolution of egg size  
34 in captive salmon. *Nature* 299: 1738-1740.

- 1 Hedrick, P., S. Hedgecock, S. Hamelberg, and S. Croci. 2000. The impacts of  
2 supplementation in winter-run Chinook salmon on effective population size.  
3 *Journal of Heredity* 91 (2): 112-115.
- 4 Hillis, D., C. Moritz and B. Mable. 1996. Nucleic acids III: sequencing. Pages 205-247 *in*  
5 Hillis, D., Moritz, C. and Mable, B., editors. *Molecular Systematics*. Sinauer.
- 6 Hindar, K., N. Ryman, and F. Utter. 1991. Genetics effects of cultured fish on natural  
7 populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 945-957.
- 8 Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide  
9 sequences. *Proceedings of the National Academy of Sciences USA* 78:454-458.
- 10 Lynch, M., and M. O’Hely. 2001. Captive breeding and genetic fitness of natural  
11 populations. *Conservation Genetics* 2: 363-378.
- 12 Lacy, R. 1987. Loss of genetic diversity from managed populations: interacting effects of  
13 drift, mutation, selection and population subdivision. *Conservation Biology* 1:  
14 143-157.
- 15 Lynch, M., J. Conery, and R. Burger. 1995. Mutational accumulation and the extinction  
16 of small populations. *American Naturalist* 146: 489-518.
- 17 Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- 18 Palm, S., J. Dannewitz, T. Järvi, E. Peterson, T. Prestegard and N. Ryman. 2003. Lack of  
19 molecular genetic divergence between sea-ranched and wild sea trout (*Salmo*  
20 *trutta*). *Molecular Ecology* 12: 2057-2071.
- 21 Pfliegler, W. 1980. *Hybognathus nuchalis* Agassiz Central silvery minnow. Page 177 *in*  
22 *Atlas of North American freshwater fishes* D. S. Lee et al. editors. North Carolina  
23 State Museum Natural History, Raleigh.
- 24 Platania, S. and C. Altenbach. 1998. Reproductive strategies and egg types in seven Rio  
25 Grande basin cyprinids. *Copeia* 3: 559-569.
- 26 Platania, S. and R. Dudley. 2000. Spatial spawning periodicity of Rio Grande silvery  
27 minnow during 1999. Report to US Bureau of Reclamation, Albuquerque, New  
28 Mexico.
- 29 Propst, D. 1999. Threatened and endangered fishes of New Mexico. Technical Report  
30 Number 1. New Mexico Department of Game and Fish, New Mexico.
- 31 Ralls, K. and J. Ballou. 1983. Extinction: Lessons from zoos. Pages 164-184 In C. B.  
32 Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, eds. *Genetics*  
33 *and Conservation: a reference for managing wild animal and plant populations*.  
34 Benjamin/Cummins, Melo Park, California, USA.

- 1 Raymond, M. and F. Rousset. 1995. GENEPOP Version 1.2: population genetics  
2 software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.
- 3 Remshardt, J. 2002. Augmentation and monitoring plan for the Rio Grande silvery  
4 minnow in the middle Rio Grande, New Mexico. Region 2 U.S. Fish and Wildlife  
5 Service. Fishery Resource Office, Albuquerque New Mexico.
- 6 Ryman, N., and L. Laike. 1991. Effects of supportive breeding on the genetically  
7 effective population size. *Conservation Biology* 8: 888-890.
- 8 Ryman, N. and G. Shahl. 1980. Genetic changes in hatchery stock of brown trout (*Salmo*  
9 *trutta*). *Canadian Journal of Fisheries and Aquatic Sciences* 37: 82-87.
- 10 Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: A software for population  
11 genetic analyses. Version 2.000. Genetics and Biometry Laboratory, Department  
12 of Anthropology, University of Geneva.
- 13 Sunnucks, P., C. Wilson, L. Beheregaray, K. Zenger, J. French, and A. Taylor. 2000.  
14 SSCP is not so difficult: the application and utility of single-stranded  
15 conformation polymorphism in evolutionary biology and molecular ecology.  
16 *Molecular Ecology* 9: 1699-1710
- 17 Stockwell, C., M. Mulvey, and G. Vinyard. 1996. Translocations and the preservation of  
18 allelic diversity. *Conservation Biology*, 10 (4): 1133-1141.
- 19 Tringali, M. and T., Bert. 1998. Risk to genetic effective population size should be an  
20 important consideration in fish stock-enhancement programs. *Bulletin of Marine*  
21 *Sciences* 62 (2): 641-659.
- 22 Turner, T., T. Dowling, R. Broughton, and J. Gold. 2003. Variable microsatellite markers  
23 amplify across divergent lineages of cyprinid fishes (subfamily Leuciscinae).  
24 *Conservation Genetics*, in press.
- 25 US Fish and Wildlife Service. 2003. <http://fisheries.fws.gov/FTC/index.html>.
- 26 Wang, S., J. Hard, and F. Utter. 2002. Salmonid inbreeding: a review. *Reviews in Fish*  
27 *Biology and Fisheries* 11: 301-319.
- 28 Weir, B., and C. Cockerham. 1984. Estimating F-statistics for the analysis of population  
29 structure. *Evolution* 38: 1358-1370.
- 30

**Figure 1:** Current distribution of Rio Grande silvery minnow (middle Rio Grande New Mexico). Arrows show the approximate location of wild Rio Grande silvery minnow collection sites.



**Table 1:** Summary statistics for ten microsatellite and mtDNA – ND4 loci screened for wild Rio Grande silvery minnow collected in 2002 (Angostura, Isleta, San Acacia), hatchery-spawned and reared (ABQ BioPark) (Hatchery 2001), and hatchery-reared fish from wild-caught eggs (Hatchery 2002, Hatchery 2003). Expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), number of alleles (total number of alleles across all populations is given in parenthesis), allele size range, allelic richness and average weighted inbreeding co-efficient ( $F_{IS}$ ) (significant  $F_{IS}$  values at  $\alpha = 0.05$  are given in bold) are give for all loci. For ND4 the observed number of haplotypes and the gene diversity ( $h$ ) are given.

Locus	Population	Hatchery	Hatchery	Hatchery	Angostura	Isleta	San Acacia	Combined WILD
		2001	2002	2003				
	Sample size	64	178	81	67	121	201	389
<i>Lco1</i>	$H_E$	0.949	0.961	0.967	0.959	0.962	0.963	0.963
	$H_O$	0.821	0.577	0.722	0.862	0.775	0.838	0.823
	No Alleles (51)	26	38	36	34	38	42	47
	Size Range	241-344	201-344	209-348	221-342	201-348	205-348	201-348
	Allelic Richness	25.816	30.793	32.542	33.133	34.424	35.335	32.763
	$F_{IS}$	0.135	0.400	0.254	0.102	0.194	0.129	0.146
<i>Lco3</i>	$H_E$	0.752	0.789	0.819	0.796	0.776	0.764	0.777
	$H_O$	0.807	0.566	0.541	0.810	0.819	0.774	0.794
	No Alleles (16)	8	14	12	11	12	13	14
	Size Range	241-257	235-265	237-261	235-257	235-259	237-263	235-263
	Allelic Richness	7.895	10.600	11.490	10.690	10.392	10.362	10.646
	$F_{IS}$	-0.073	<b>0.283</b>	<b>0.341</b>	<b>-0.017</b>	<b>-0.056</b>	<b>-0.014</b>	<b>-0.022</b>
<i>Lco4</i>	$H_E$	0.561	0.567	0.626	0.683	0.670	0.663	0.684
	$H_O$	0.241	0.442	0.310	0.582	0.647	0.568	0.595
	No Alleles (13)	5	11	11	8	9	10	12
	Size Range	231-237	226-237	221-234	227-234	226-237	221-237	221-237
	Allelic Richness	5.000	8.511	9.861	7.224	7.946	7.859	8.513
	$F_{IS}$	<b>0.573</b>	<b>0.222</b>	<b>0.507</b>	<b>0.149</b>	<b>0.034</b>	<b>0.144</b>	<b>0.13</b>

<b>Lco5</b>	$H_E$	0.418	0.558	0.520	0.699	0.626	0.509	0.593
	$H_O$	0.458	0.672	0.529	0.587	0.436	0.406	0.446
	No Alleles (8)	4	5	4	5	6	8	8
	Size Range	130-133	129-133	130-133	130-134	129-134	129-136	129-136
	Allelic Richness	3.915	4.280	3.999	4.981	5.449	6.770	5.642
	$F_{IS}$	-0.095	<b>-0.205</b>	<b>-0.017</b>	<b>0.160</b>	<b>0.305</b>	<b>0.203</b>	<b>0.249</b>
	<b>Lco6</b>	$H_E$	0.651	0.818	0.781	0.745	0.650	0.626
$H_O$		0.696	0.475	0.456	0.524	0.504	0.434	0.472
No Alleles (26)		12	24	18	18	17	20	23
Size Range		168-189	163-187	166-189	164-189	162-189	166-189	162-189
Allelic Richness		11.924	18.570	16.225	17.082	13.956	15.525	17.356
$F_{IS}$		-0.071	<b>0.420</b>	<b>0.418</b>	<b>0.298</b>	<b>0.225</b>	<b>0.307</b>	<b>0.28</b>
<b>Lco7</b>		$H_E$	0.818	0.884	0.883	0.809	0.824	0.797
	$H_O$	0.625	0.512	0.329	0.469	0.537	0.548	0.531
	No Alleles (24)	10	22	18	13	14	18	19
	Size Range	137-163	137-169	137-164	141-169	137-169	137-169	137-169
	Allelic Richness	9.962	16.209	17.000	12.325	12.084	13.385	16.667
	$F_{IS}$	<b>0.238</b>	<b>0.422</b>	<b>0.629</b>	<b>0.423</b>	<b>0.349</b>	<b>0.313</b>	<b>0.345</b>
	<b>Lco8</b>	$H_E$	0.788	0.882	0.857	0.846	0.884	0.877
$H_O$		0.625	0.425	0.444	0.613	0.575	0.633	0.612
No Alleles (27)		14	20	12	14	17	20	21
Size Range		274-312	254-310	272-312	274-310	274-312	270-310	270-312
Allelic Richness		13.325	15.905	10.663	13.578	14.412	14.364	14.967
$F_{IS}$		<b>0.208</b>	<b>0.519</b>	<b>0.483</b>	<b>0.277</b>	<b>0.350</b>	<b>0.278</b>	<b>0.303</b>
<b>CAI</b>		$H_E$	0.547	0.531	0.366	0.460	0.366	0.252
	$H_O$	0.156	0.165	0.050	0.164	0.033	0.131	0.106
	No Alleles (18)	4	13	6	8	8	7	11
	Size Range	75-91	74-96	75-92	75-95	75-95	75-95	75-95
	Allelic Richness	3.844	8.671	5.142	7.03	5.918	4.977	7.184
	$F_{IS}$	<b>0.716</b>	<b>0.690</b>	<b>0.864</b>	<b>0.644</b>	<b>0.910</b>	<b>0.482</b>	<b>0.678</b>

<b>CA6</b>	$H_E$	0.685	0.828	0.750	0.805	0.811	0.741	0.786
	$H_O$	0.781	0.638	0.638	0.821	0.736	0.658	0.711
	No Alleles (22)	10	18	11	11	14	16	19
	Size Range	201-221	191-225	195-217	185-221	191-223	189-221	185-223
	Allelic Richness	9.614	14.155	10.526	10.418	11.406	10.964	12.579
	$F_{IS}$	<b>-0.142</b>	<b>0.230</b>	<b>0.150</b>	<b>-0.020</b>	<b>0.093</b>	<b>0.111</b>	<b>0.096</b>
	<b>CA8</b>	$H_E$	0.935	0.944	0.946	0.934	0.946	0.965
$H_O$		0.491	0.536	0.516	0.667	0.740	0.663	0.689
No Alleles (36)		20	28	26	21	28	33	34
Size Range		100-216	100-222	100-216	104-222	104-222	104-228	104-228
Allelic Richness		19.887	22.914	25.057	21.000	24.394	26.294	25.145
$F_{IS}$		0.475	0.432	0.454	0.286	0.218	0.302	0.272
<b><math>F_{IS}</math> All</b>		<b>0.200</b>	<b>0.345</b>	<b>0.416</b>	<b>0.209</b>	<b>0.228</b>	<b>0.212</b>	<b>0.222</b>
<b>ND4</b>	No Alleles (11)	5 (58)	8 (157)	8 (81)	6 (68)	7 (109)	7 (200)	8 (377)
	Gene Diversity ( $h$ )	0.460	0.624	0.703	0.681	0.670	0.613	0.641

**Table 2.** Summary statistics for microsatellite and mtDNA – ND4 loci screened for wild Rio Grande silvery minnows sampled in 2002, hatchery-spawned and reared (ABQ BioPark) (Hatchery 2001), and hatchery-reared fish from wild-caught eggs (Hatchery 2002, Hatchery 2003). Sample size ( $n$ ), expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), mean number of alleles per locus, mean allelic richness (based on the minimum sample size of 58) and average weighted inbreeding co-efficient ( $F_{IS}$ ) are give over all loci. For ND4 the observed number of haplotypes and the gene diversity ( $h$ ) are given

Statistics	Population			
	Hatchery 2001	Hatchery 2002	Hatchery 2003	<b>Wild</b>
<b><i>Microsatellites</i></b>				
$n$	64	178	81	389
$H_E$	0.708	0.760	0.761	0.740
$H_O$	0.567	0.498	0.446	0.575
Alleles/Locus	11.00	18.50	15.10	20.20
Allelic Richness	10.820	14.383	13.961	14.722
$F_{IS}$	0.200	0.345	0.416	0.222
<b><i>MtDNA-ND4</i></b>				
$n$	58	157	81	377
$h$	0.460	0.624	0.703	0.641
Haplotypes	5	8	8	8

**Table 3.** Mitochondrial ND4 haplotype frequencies among wild Rio Grande silvery minnows sampled in 2002, Hatchery-spawned and reared (ABQ BioPark) (Hatchery 2001), and hatchery-raised fish from wild-caught eggs (Hatchery 2002, Hatchery 2003).

Haplotype	Hatchery 2001	Hatchery 2002	Hatchery 2003	Wild 2002
A	0.724	0.573	0.481	0.541
C	0.052	0.197	0.222	0.204
D	-	0.051	0.049	0.357
E	0.034	0.064	0.012	0.012
F	0.069	0.064	0.136	0.058
J	-	-	-	0.003
K	0.121	0.032	0.049	0.032
M	-	0.013	0.037	-
N	-	0.006	-	-
O	-	-	-	0.003
P	-	-	0.012	-