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Article *in* Reviews in Fisheries Science · January 2006

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To cite this article: Megan J. Osborne , Melissa A. Benavides , Dominique Alò & Thomas F. Turner (2006) Genetic Effects of Hatchery Propagation and Rearing in the Endangered Rio Grande Silvery Minnow, Hybognathus amarus , Reviews in Fisheries Science, 14:1-2, 127-138, DOI: 10.1080/10641260500341544

To link to this article: http://dx.doi.org/10.1080/10641260500341544

Published online: 18 Jan 2007.

Reviews in Fisheries Science, 14:127–138, 2006

ISSN: 1064-1262 print
DOI: 10.1080/10641260500341544

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Genetic Effects of Hatchery Propagation and Rearing in the Endangered Rio Grande Silvery Minnow, *Hybognathus amarus*

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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 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.*

Keywords captive propagation, microsatellite loci, mitrochondrial DNA ND4, allelic diversity, heterozygosity

Introduction

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

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

Although adult census size of the wild population may be increased by supportive breeding and subsequent introductions, there are risks associated with such measures. Detrimental genetic impacts include introduction of nonadaptive traits (Lynch et al., 1995; Heath et al., 2003), reduction in the effective population size (Ryman and Laike, 1991), inbreeding depression (Frankham, 1995), and maladaptive behavioral changes (Hindar et al., 1991). Hatchery-reared fish may be depauperate of overall genetic diversity and this deficiency may ultimately reduce variability in wild recipient populations (Tringali and Bert, 1998). This will be most evident if few founders are used as brood stock. For a hatchery population to retain 99% of the heterozygosity of the wild population, 50–500 effective founding breeders has been recommended (Ryman and Stahl; 1980; Frankel and Soulé, 1981; Frankham, 1995). A small brood stock is expected to lose heterozygosity and exhibit lowered viability and fecundity as a result of inbreeding depression (Falconer, 1981; Ralls and Ballou, 1983).

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

Materials and Methods

Sampling Localities and Methods

Wild *Hybognathus amarus* were sampled from the middle Rio Grande, New Mexico. Three water-diversion structures (from north to south—Angostura Diversion Dam, Isleta Diversion Dam, and San Acacia Diversion Dam) divide the middle Rio Grande into four reaches: (1) Cochiti [36 kms] (2) Angostura [65 kms], (3) Isleta [86 kms], and (4) San Acacia [92 kms]. The present study focuses on the latter three reaches because *H. amarus* are now extremely rare in the Cochiti reach (Bestgen and Platania, 1991). Wild adult *H. amarus* were collected prior to spawning (December 2001 through March 2002) by seining and backpack electrofishing. Captured fishes were anesthetized in MS-222 (Tricaine Methane

Sulfonate 200 mg/L river water) at the capture site and a small piece of caudal fin was removed from each individual ($n = 389$). Fish were allowed to recover in untreated river water prior to release.

Three-year classes of hatchery-propagated *H. amarus* were considered. Year 2001, fishes (referred to hereafter as Hatchery 2001) were raised from captively-spawned wild caught adults (collected from the San Acacia reach in 2000). It is unknown how many brood stock were used in captive spawning. Collection of eggs for propagation activities were made during the peak spawning period that occurred from 8–11 May 2001 (Hatchery 2002 sample) and 17–19 May 2002 (Hatchery 2003 sample). Approximately 100,000 and 922,000 eggs were collected in 2001 and 2002, respectively, by the U.S. Fish and Wildlife Service. Collections of drifting eggs occurred 16 km downstream of the San Marcial railroad bridge (Socorro County) in the San Acacia reach of the Rio Grande using modified Moore egg collectors (Altenbach et al., 2000). Eggs were raised in propagation facilities and fin clips were taken from these fish (Hatchery 2002 and Hatchery 2003 samples) prior to their release and stored in 95% ethanol. DNA was extracted from air-dried fin clips using standard proteinase-k digestion and organic extraction methods (Hillis et al., 1996).

Characterization of Genetic Diversity: Microsatellites

Individuals were screened for genetic variation at 10 microsatellite loci: *Lco1*, *Lco3*, *Lco4, Lco5, Lco6, Lco7*, and *Lco8* (Turner et al., 2004) and *CA1, CA6*, and *CA8* (Dimsoski et al., 2000). Microsatellite loci were visualized using fluorescently labeled forward primers. The following microsatellites were amplified (in a 10 μL reaction volume) using multiplex PCR: *Lco3, Lco4,* and *Lco5* (1X PCR buffer, 2 mM $MgCl_2$, 200 mM dNTPs, 0.40 μM each primer, 0.375 units TAQ polymerase); *Lco6* and *Lco7* (1X PCR buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.40 μM each primer, 0.375 units TAQ polymerase); *CA1* and *CA6* (1X PCR buffer, 2 mM $MgCl_2$, 200 mM dNTPs, 0.40 μM each primer, 0.375 units TAQ polymerase). The remaining microsatellites were amplified alone (*Lco1*, *Lco8*, and *CA8*) (1X PCR buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.50 μM each primer, 0.375 units TAQ polymerase). Polymerase chain reaction (PCR) cycling conditions were: one denaturation cycle of 94°C for 2 min followed by 30 cycles of 94°C for 20 s, 48°C (*Lco6*, *Lco7*, *CA1*, *CA6*) or 50°C (*Lco3*, *Lco4*, *Lco5*, *Lco8*) or 52°C (*Lco1*, *CA8*) for 20 sec, 72°C for 30 s. Prior to electrophoresis 1.2 μL of PCR product was mixed with 1.2 μL of a solution containing 62.5% formamide, 25% bromophenol blue, 12.5% Genescan ROX350 (ABI PRISM, Applied Biosystems, California, USA) size standard and denatured at 94°C for 2 min and placed on ice. Products were electrophoresed in an ABI377 Prism (Applied Biosystems) automated sequencer and analyzed with GeneScan Version 3.1.2 (Applied Biosystems) software.

MtDNA-ND4

A 295 base-pair fragment of the mitochondrial ND4 gene was amplified (10 μL reaction) using the following conditions: 1 μL DNA (50–100 ng/μL), 1X reaction buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.50 μM forward (5′-GAC CGT CTG CAA AAC CTT AA-3′) and reverse primer (5′-GGG GAT GAG AGT GGC TTC AA-3′), 0.375 units TAQ polymerase. PCR parameters were initial denaturation of 94°C for 2 min followed by 30 cycles of 94°C for 30s, 52°C for 30s and 72°C for 30s. Single-stranded conformational polymorphism (SSCP) (Sunnucks et al., 2000) was used to characterize the genetic diversity in *H. amarus*. To confirm haplotype designations, a proportion of variants were sequenced from each

gel using a ABI BigDye Terminator cycle sequencing (ABI, California, USA) kit and an ABI377 Prism automated sequencer. Sequencher Version 4.1.2 (Gene Codes Corporation, Michigan, USA) software was used to read sequences.

Data Analysis

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

Analyses of Population Structure

For microsatellites and mtDNA-ND4 hierarchical analysis of molecular variance (AMOVA) was used to partition standardized genetic variance into differences among groups (two groups - hatchery stocks and wild population) (F_{CT}); differences between populations within groups (F_{SC}); and among all populations (F_{ST}). Weir and Cockerham's (1984) F-statistics were obtained using AMOVA as implemented in Arlequin (Schneider et al., 2000).

Results

Genetic Diversity - Microsatellites

There were between eight (*Lco5*) and 61 (*Lco1*) alleles in the 10 loci considered (Table 1). Allelic richness ranged from 3.844 (*CA8*) to 35.824 (*Lco1*) (Table 1). With the exception of *Lco8*, allelic richness was lowest at all loci for the Hatchery 2001 population. Significant linkage disequilibrium was identified between a single pair of loci *Lco6* and *Lco7* ($P < 0.001$). In the Hatchery 2001 population, allele frequencies at *Lco3*, *Lco5*, *Lco6*, and *CA6* did not differ significantly from Hardy-Weinberg expectation (Table 1). The remaining loci in the Hatchery 2001 population and all loci in the remaining populations (San Acacia, Isleta, Angostura, Hatchery 2002, and Hatchery 2003) deviated from Hardy-Weinberg expectations ($P < 0.01$). A global test revealed a deficiency of heterozygotes for all populations and loci ($P < 0.0001$). Over all loci, F_{IS} values ranged from 0.199 (Hatchery 2001) to 0.415 (Hatchery 2003) (Table 2). F_{IS} for the Hatchery 2003 stock was twice that in the wild population (0.223) (Table 2).

Mitochondrial DNA - ND4

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

Table 1

Summary statistics for 10 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 given for all loci. For ND4, the observed number of haplotypes and the gene diversity (h) are given

Locus	Population	Hatchery 2001	Hatchery 2002	Hatchery 2003	Angostura	Isleta	San Acacia	Combined Wild
	Sample size	64	178	81	67	121	201	389
Lco1	H_E	0.948	0.962	0.965	0.959	0.964	0.965	0.965
	H_O	0.821	0.576	0.722	0.862	0.775	0.838	0.822
	No alleles (61)	27	41	36	35	42	50	55
	Size range	241–344	201–344	209–348	221–342	201–348	205–348	201–348
	Allelic richness	26.745	32.733	32.542	33.133	34.424	35.335	35.824
	F_{IS}	**0.135**	**0.401**	**0.254**	**0.102**	**0.197**	**0.132**	**0.148**
Lco3	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	**0.283**	**0.341**	**−0.017**	**−0.056**	**−0.014**	**−0.022**
Lco4	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}	**0.573**	**0.222**	**0.507**	**0.149**	**0.034**	**0.144**	**0.13**
Lco5	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	**−0.205**	**−0.017**	**0.160**	**0.305**	**0.203**	**0.249**
Lco6	H_E	0.651	0.818	0.781	0.745	0.650	0.626	0.655
	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	**0.420**	**0.418**	**0.298**	**0.225**	**0.307**	**0.28**
Lco7	H_E	0.818	0.884	0.883	0.809	0.824	0.797	0.811
	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}	**0.238**	**0.422**	**0.629**	**0.423**	**0.349**	**0.313**	**0.345**
Lco8	H_E	0.788	0.882	0.857	0.846	0.884	0.877	0.877

(Continued on next page)

Table 1

Summary statistics for 10 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 given for all loci. For ND4 the observed number of haplotypes and the gene diversity (h) are given *(Continued)*

Locus	Population	Hatchery 2001	Hatchery 2002	Hatchery 2003	Angostura Angostura	Isleta	San Acacia	Combined WILD
	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}	**0.208**	**0.519**	**0.483**	**0.277**	**0.350**	**0.278**	**0.303**
CA1	H_E	0.547	0.531	0.366	0.460	0.366	0.252	0.329
	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}	**0.716**	**0.690**	**0.864**	**0.644**	**0.910**	**0.482**	**0.678**
CA6	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}	−0.142	**0.230**	**0.150**	**−0.020**	**0.093**	**0.111**	**0.096**
CA8	H_E	0.938	0.959	0.944	0.938	0.948	0.967	0.958
	H_O	0.491	0.542	0.516	0.667	0.739	0.663	0.689
	No alleles (60)	24	38	28	26	32	53	57
	Size range	101–217	101–222	101–218	104–222	104–222	104–229	104–229
	Allelic richness	23.778	29.870	26.798	26	26.496	38.505	32.957
	F_{IS}	**0.475**	**0.432**	**0.455**	**0.286**	**0.218**	**0.302**	**0.2721**
F_{IS} All		**0.199**	**0.356**	**0.398**	**0.213**	**0.229**	**0.211**	**0.223**
ND4	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

Population Structure - Microsatellites

Standardized genetic variance attributable to differences among river reaches was $F_{ST} = 0.0138$ ($P < 0.001$) and among the three hatchery populations was $F_{ST} = 0.0153$ ($P < 0.001$). A significant proportion of genetic variation was explained by differences between wild and hatchery populations ($F_{CT} = 0.009$, $P < 0.001$).

Mt-DNA ND4

Significant genetic variation was attributable to differences among populations within groups (hatchery and wild) ($F_{SC} = 0.010$, $P = 0.003$). No significant variation was

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

	Population			
Statistics	Hatchery 2001	Hatchery 2002	Hatchery 2003	Wild
Microsatellites				
n	64	178	81	389
H_E	0.711	0.778	0.751	0.744
H_O	0.570	0.502	0.453	0.578
Alleles/Locus	11.80	20.60	15.60	23.90
Allelic Richness	11.60	15.95	14.43	15.47
MtDNA-ND4				
F_{IS}	0.199	0.356	0.398	0.223
n	58	157	81	377
h	0.460	0.624	0.703	0.641
Haplotypes	5	8	8	8

attributable to differences between hatchery and wild populations ($F_{CT} = 0.006$, $P = 0.100$). Significant variation was explained by differences among the three hatchery stocks (2001, 2002 and 2003) ($F_{ST} = 0.026$, $P = 0.004$). No significant genetic variation was explained by differences among the three river reaches ($F_{ST} = 0.003$, $p = 0.644$).

Discussion

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

Comparison of Wild and Captively Propagated Stocks (Hatchery 2001)

Observed heterozygosity of the captively spawned population (Hatchery, 2001) is equivalent to that seen in the wild population, but allelic diversity is much lower. The loss of alleles and haplotypes from captively-spawned *H. amarus* is not surprising as most rare alleles will not be sampled when the brood stock is founded by relatively few individuals. If rare alleles are sampled, they are likely to be lost rapidly by genetic drift (Lacy, 1987) as the probability of retention is directly proportional to the effective population size, N_e (Allendorf, 1986). For the captive brood stock obtained in year 2000 (Hatchery, 2001), N_e is roughly equal

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	—

to the number of breeders that actually contributed offspring to the subsequent generation. Lowered allelic diversity in captively spawned stocks is consistent with the observation that rare alleles are lost more rapidly than heterozygosity when N_e is reduced substantially (as in a "genetic bottleneck" event) (Lacy, 1987), which implies that N_e was smaller in the captive brood stock than in the wild source population.

The detection of very few, closely related mitochondrial ND4 haplotypes in the 670 *H. amarus* screened is consistent with a population that has experienced bottleneck events in the recent past (Avise, 2000). During bottleneck events, the probability of retaining an allele is directly proportional to its frequency in the population (Allendorf, 1986); hence, rare alleles are more likely to be lost during such events. If severe population reductions occur in the northern reaches of the Rio Grande they can only be recolonized by artificial translocations of individuals as diversion structures prevent upstream movement.

Hatchery-Reared Progeny of Wild-Caught Eggs

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

At several loci, allelic richness in hatchery (2002, 2003) stocks actually exceeded that observed in the wild population. The pelagic nature of *H. amarus* eggs means that a substantial proportion of eggs are likely to be transported to unsuitable nursery habitats where they are subject to heavy mortality. It is expected that only a small fraction of the total spawn will be successfully recruited into the adult population (Alò and Turner, 2005). Survival of eggs and subsequent early life stages is greater in propagation facilities than

in the wild population. Increased allelic richness in hatchery stocks (2002, 2003) might be explained by a reduction of variance in mortality of the hatchery stocks.

Implications for Species Recovery

Hybognathus amarus is a short-lived fish with few individuals surviving beyond 13 months in the wild (Propst, 1999). The impact of supplementation is likely to be more extreme and rapid in *H. amarus* than in long-lived species because repatriated hatchery-reared fish can contribute immediately to the reproductive output of the recipient wild population. Theory indicates that when captively reared fish represent a large proportion of the total number of breeders in the wild population, effective population size is reduced in subsequent generations (Ryman and Laikre, 1991). This will be exacerbated if the reproductive contribution among brood stock individuals is unequal due to factors such as incomplete mixing of sperm and eggs, sperm competition, variation in female fecundity, and differential survival of matings (Brown et al., 2000). This would result in a lower than predicted N_e in the captive population. Selecting brood stock from sampling localities throughout the species distribution, maximizing the number of brood stock used, and equalizing the reproductive contributions of individuals could help to reduce, but not eliminate, loss of genetic variation in captively spawned fish.

Hatchery stocks from wild caught eggs in 2002 and 2003 retain only about 78% of the heterozygosity of the parental source population. This trend in the captively propagated *H. amarus* is a concern given that from the 2003 captive stock alone, 130,000 fish have been repatriated to the Rio Grande. If these fish spawn successfully, then theory predicts increasingly high F_{IS} values will be apparent in subsequent generations. Genetic effective size of the wild population of *H. amarus* is already very small ($N_e \approx 70$) (Alo and Turner, 2005), and problems associated with small effective size of the wild population may be compounded by supplementation. For example, loss of heterozygosity and allelic diversity in captive or refugial populations has been reported in a large number of fish species including the mosquito fish (*Gambusia affinis*) (Stockwell et al., 1996), cutthroat trout (*Onchorhynchus clarki*) (Allendorf and Phelps, 1980), and Atlantic salmon (*Salmo salar*) (Cross and King, 1983). There are also numerous examples where gene frequencies have shifted in the wild population to resemble those of the hatchery stocks (Altukhov, 1981). Shifts in allele frequencies (towards those seen in the hatchery stocks) can be expected in the wild population of *H. amarus* especially with a continued decline in the number of wild fish and continued release of hatchery fish.

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

Although introduction of hatchery-raised fish may temporarily increase adult census population size, the status of the wild population of *H. amarus* is unlikely to be improved

unless the primary reasons for the populations continued decline are addressed. Our data indicate that captive propagation, hatchery rearing, and supplementation of wild populations is likely to lead to genetic changes that will decrease the probability of long-term persistence of *H. amarus* in the wild. Our recommendation is that propagation and supplementation be used sparingly as a tool to prevent extinction from catastrophic events, but not as a panacea for the long-term conservation of *H. amarus*.

Acknowledgments

Our sincere thanks are extended to S. P. Platania (SPP), R. K. Dudley, W. H. Brandenburg, A. M. Snyder, M. A. Farrington, C. S. Altenbach, M. D. Porter, J. E. Brooks, D. L. Propst, M. Ulibarri, the Albuquerque Fishery Resources Office of the U.S. Fish and Wildlife Service, and the Museum of Southwestern Biology for technical and logistic support throughout the project. G. Rosenburg and the staff of the UNM Molecular Biology Core Facility provided vital technical support. Funding was provided by New Mexico Department of Game and Fish, the U.S. Forest Service, the U.S. Bureau of Reclamation, the U.S. Fish and Wildlife Service, and the National Science Foundation. The manuscript benefited from comments by G. Moyer and two anonymous reviewers. Rio Grande silvery minnow were collected under Federal Fish and Wildlife Permits TE001623-0 (SPP) and TE038055-0 (TFT) and the New Mexico Department of Game and Fish Scientific Collecting Permits 1896 (SPP) and 3015 (TFT).

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