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Genetic heterogeneity among pelagic egg samples and variance in reproductive success in an endangered freshwater fish, *Hybognathus amarus* (Cyprinidae)

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Synopsis

A sweepstakes–mismatch process whereby reproduction is poorly coordinated with appropriate resources for larval development and recruitment can result in large variance in reproductive success among individuals and spawning aggregations. This process has been proposed to explain low ratio of genetic effective population size (N_e) to adult census size (N) ratios in marine species with high fecundity, pelagic spawning, and extensive mortality in early life stages. This process is also hypothesized to also account for very low N_e/N ($\approx$ 0.001) observed in the federally endangered Rio Grande silvery minnow, *Hybognathus amarus*. This species is a freshwater fish that shares life-history features with marine pelagic spawners. We tested two key predictions of the sweepstakes–mismatch hypothesis using molecular data: (i) that temporally distinct samples of eggs differ in genetic composition and, (ii) that egg samples do not comprise a random subset of potential adult breeders. We present genetic data that supports both predictions and that are consistent with the hypothesis that high variance in reproductive success among adult breeders is an important factor that lowers N_e/N in *H. amarus*. This study highlights the importance of understanding the interaction of early life history and fragmentation in devising conservation plans for endangered aquatic organisms.

Introduction

Although remarkably tolerant of severe environmental conditions, freshwater fishes of the southwestern United States have suffered declines in abundance, local extirpation and extinction as human demand for water has increased in this arid region (Minckley & Deacon 1991, Minckley et al. 2003). River fragmentation by impoundments and large-scale diversion of water are implicated as major factors driving species decline and extinction (Minckley & Deacon 1968, Moyle & Williams 1990, Winston et al. 1991). The federally endangered (United States Department of the Interior 1994) Rio Grande silvery minnow, *Hybognathus amarus* (Cyprinidae), is a case in point. Historically, this species was abundant and widely distributed throughout the Rio Grande basin in New Mexico and Texas (USA) and in northern Mexico (Trevino-Robinson 1959). Now however, *H. amarus* occupies only 5% of its historical range and is restricted to a 280-km river reach in New Mexico where the river is fragmented by three water diversion dams (Angostura, Isleta, San Acacia) and two major storage dams (Cochiti, Elephant Butte) (Figure 1).

H. amarus produce pelagic eggs that drift downstream with river currents (Platania & Altenbach 1998); an early life-history feature that is not commonly observed in river fishes. Most lotic species

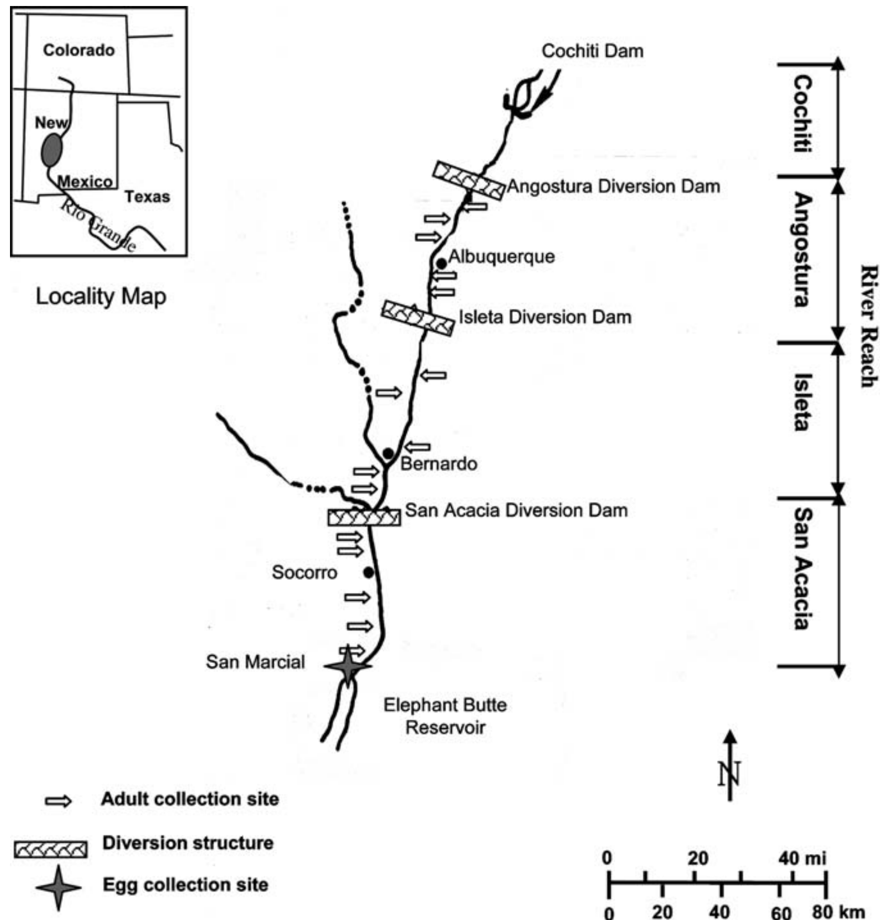

Figure 1. Map of the current distribution of *H. amarus* (inset), wild adult monitoring/collection sites (arrows) and egg collection site (star) in the middle Rio Grande, New Mexico. The four river reaches delimited by three water diversion structures are also shown.

produce demersal (sinking) or adhesive eggs, ostensibly as a strategy to retain eggs in upstream portions of a flowing stream. Pelagic spawning probably was once adaptive in the turbid waters of the Rio Grande (Moore 1944) because demersal or adhesive eggs would quickly become covered by silt and fail to develop.

Pelagic early life-history has been implicated, in part, for the decline of *H. amarus* in the now extensively fragmented Rio Grande. At present drifting eggs and larvae are subject to entrainment through diversion structures and dams, movement from natal sites and presumably heavy mortality as they are transported into unfavorable nursery habitats such as Elephant Butte Reservoir (Platania & Altenbach 1998, Luttrell et al. 1999). Dams and impoundments prevent upstream migration by adults and such adults may perish when downstream reaches dry in summer.

A previous genetic study of *H. amarus* revealed genetic consequences of river fragmentation that mirrored demographic consequences, namely that variance genetic effective size (N_e) of the largest remnant population is roughly three orders of magnitude smaller than estimates of adult census size (N) (Alò & Turner 2004). In pelagic-spawning marine fishes the 'sweepstakes–mismatch' process was initially proposed to explain low N_e/N in these species (Hedgecock 1994, Li & Hedgecock 1998). The sweepstakes–mismatch process may strongly affect N_e/N in *H. amarus* through an interaction of pelagic life-history, large-scale water diversion, and habitat fragmentation by dams (Alò & Turner 2004) resulting in heavy mortality of early life stages. In principle, the effect on N_e/N is maximal if groups of eggs spawned at different places and times are genetically divergent as a result of being

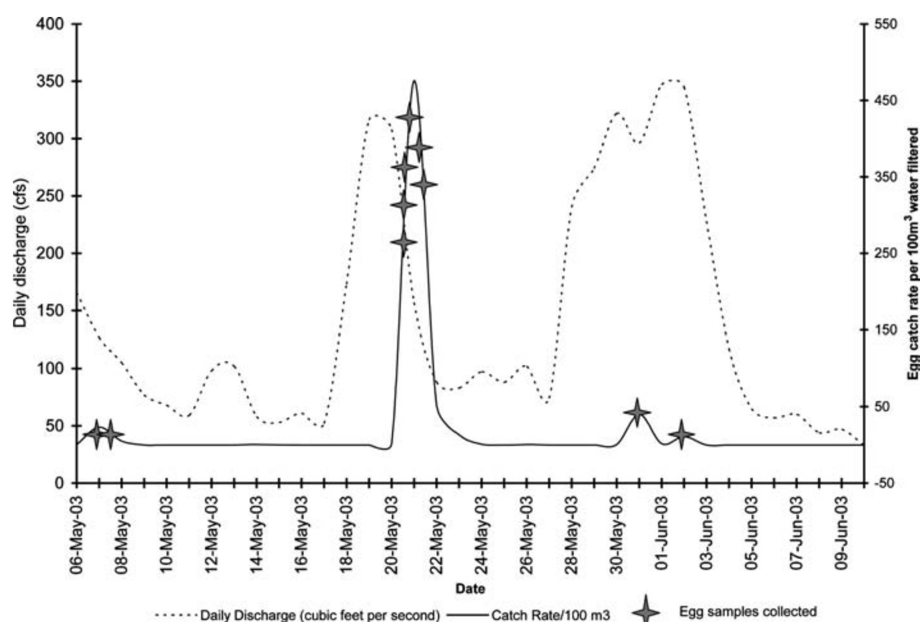

Figure 2. Mean daily discharge is indicated by a dashed line (cubic feet per second [cfs] at USGS Gauge 08358400). *H. amarus* egg catch rate (catch per unit effort of drifting eggs was calculated as the total number of eggs collected × volume of water sampled^{-1} × 100 [Platania and Dudley 2004 unpublished]) at the San Marcial egg collection site is shown by the solid line. Stars indicate the egg collections used for genetic analysis.

produced by small groups of breeders. In this scenario whole groups of closely related progeny are subject to severe, but differential mortality (i.e. family correlated survival – Waples 2002).

We tested two null hypotheses using genetic data to evaluate the efficacy of the sweepstakes–mismatch hypothesis for explaining low N_e/N observed in *H. amarus*: (i) eggs do not differ in genetic composition among samples collected at a single location during the reproductive season in 2003 and (ii) gene frequencies do not differ between egg samples and prospective breeding adults. Rejection of these would imply that a sweepstakes–mismatch process is important in lowering N_e/N in *H. amarus*.

Materials and methods

Sampling for genetic analysis

We collected *H. amarus* eggs at a single site, located approximately 16 kms downstream of the San Marcial railroad bridge crossing in the San Acacia reach of the middle Rio Grande (Socorro County, River mile 58.8; UTM 307846 easting, 3716150 Northing, Zone 13 – Figure 1). Sampling took place from 5 May through 1 June 2003 and encompassed the entire spawning season (Figure 2). Spawning appears cued to immediately follow high river flows or ‚flow spikes' in May and early June when river discharge increases from spring snowmelt and rainstorm events (Platania & Altenbach 1998). In 2003, drought conditions prompted managers to release water from upstream reservoir storage to create an artificial flow spike with the intention of inducing a spawning response in *H. amarus*. Prior to and following the release, two smaller spawning events were associated with rainstorms. Eggs were obtained eggs using modified Moore egg collectors (Altenbach et al. 2000). We sub-sampled a total of 450 eggs from the collection (approximately 40 eggs from 10, 1-h time blocks during three distinct bouts of spawning; Table 1, Figure 2.)

We also took genetic samples of potential adult spawners for comparison to egg samples. We collected wild, reproductively capable adults (n = 168) from the three river reaches (north to south:

Table 1. Summary statistics for microsatellite and mtDNA–ND4 loci screened from *H. amarus* eggs and adults.

Microsatellites									Mt-DNA	
Sample	Collection date	Minutes sampled[1]	N	H_E	H_O	A_R	F_{IS}	P	H	Haplotypes
Eggs-003	5/6/03	210	32	0.719	0.586	9.052	0.187	0.0001*	0.574	6
Eggs-004	5/7/03	240	25	0.734	0.642	8.554	0.128	0.0015	0.514	5
Eggs-039	5/20/03	60	38	0.769	0.644	10.656	0.165	0.0001*	0.536	6
Eggs-043	5/20/03	60	43	0.727	0.663	9.493	0.090	0.0022	0.378	7
Eggs-047	5/20/03	60	39	0.778	0.742	10.807	0.047	0.0557	0.601	6
Eggs-051	5/20/03	60	32	0.770	0.716	10.335	0.071	0.0165	0.578	4
Eggs-055	5/21/03	60	29	0.726	0.665	9.574	0.085	0.0097	0.639	7
Eggs-059	5/21/03	60	36	0.722	0.565	9.089	0.221	0.0001*	0.679	6
Eggs-087	5/30/03	30	40	0.709	0.619	9.826	0.129	0.0007	0.660	7
Eggs-011/102	6/1/03	240	40	0.744	0.801	9.657	−0.077	0.9953	0.537	7
Hatch	12/02	–	81	0.802	0.482	11.215	0.401	0.0001*	0.703	8
Wild	12/02-3/03	–	152	0.755	0.766	9.706	−0.015	0.8564	0.495	7

[1]Data provided by S. P Platania 2003 Rio Grande silvery minnow spawning periodicity study.
Sample (egg collection number ACC2003-V:7 – Museum Southwestern Biology, University of New Mexico), collection date, total minutes sampled for egg collection, sample size (N), expected heterozygosity (H_E), observed heterozygosity (H_O), mean allelic richness (A_R) (based on the minimum sample size of 22), average weighted inbreeding co-efficient (F_{IS}) and associated P-value are give over all loci. For ND4, the gene diversity (h) and observed number of haplotypes are provided. Asterisks indicate significant departures from Hardy–Weinberg equilibrium (after Bonferroni adjustment for multiple tests).

Angostura, Isleta, and San Acacia) by seining, occasionally with backpack electrofishing, between December 2002 and March 2003. We anesthetized fish (MS – 222) at the collection site and removed a small piece of caudal fin and stored them in 95% ethanol. We placed fish in river water to recover prior to release. Fin clips were also obtained from hatchery-reared *H. amarus* (Hatchery, n = 81) before repatriation into the Angostura Reach in December 2002 and March 2003. These adults were reared at a propagation facility (Dexter National Fish Hatchery and Technology Center) from eggs salvaged in May 2002 in the San Acacia reach.

We isolated genomic DNA from individual fertilized eggs by mechanically rupturing egg membranes and then suspending them in 25 μl of sterile H_2O and from fin clips using the protocol outlined in Alò & Turner (2004). We screened all samples for genetic variation at six variable microsatellite loci (*Lco1, Lco3, Lco4, Lco6, Lco7* [Turner et al. 2004]), and *CA6* (Dimsoski et al. 2000). With the exception of Lco1 (tetra-) microsatellites were dinucleotide repeats. The following microsatellite loci were amplified in 10 μl reactions using multiplex PCR: *Lco3, Lco4* (1X PCR buffer, 2 mM $MgCl_2$, 125 μM dNTPs, 0.40 μM each primer, 0.375U TAQ DNA polymerase), *Lco6, Lco7* (1 × PCR buffer, 2.5 mM $MgCl_2$, 125 μM dNTPs, 0.40 μM each primer, 0.375 U TAQ polymerase), *Lco1*, *CA6* (1 × PCR buffer, 2.5 mM $MgCl_2$, 125 μM dNTPs, 0.40 μM each primer, 0.375 U TAQ polymerase). PCR cycling conditions were: initial denaturation cycle of 94°C for 2 min, followed by 30 cycles of 94°C 20 sec, 50°C (*Lco3, Lco4, Lco6, Lco7*) or 52°C (*Lco1, CA6*) 20 s, 72°C 30 s. Microsatellite loci were amplified using fluorescein labeled primers and detected and scored using an ABI377 automated sequencer with Genescan software.

We characterized genetic variation in a 295 base pair (bp) fragment of the protein-encoding mitochondrial ND4 gene. PCR amplification and genetic characterization followed Alò & Turner (2004) and allele variation was resolved by single-strand conformational polymorphism (SSCP) analysis (Sunnucks et al. 2000) and nucleotide sequencing. Nucleotide sequences of all ND4 haplotypes were accessioned into the GenBank database under sequential accession numbers AY536873 to AY 536885 and AY 682043 to AY 682045.

Data analysis

For each microsatellite locus and sampling locality, we used FSTAT Version 2.9.3.2 (Goudet

Table 2. Pairwise F_{ST} (below diagonal, calculated from microsatellite data) and ϕ_{ST} -values (above diagonal, calculated from ND4 data) among egg samples, wild populations (Angostura [Ang], Isleta [Isl] and San Acacia [SA]) and the hatchery-reared adults.

	Eggs–003	*Eggs–004*	*Eggs–039*	*Eggs–043*	*Eggs–047*	*Eggs–051*	*Eggs–055*	*Eggs–059*	*Eggs–087*	*Egg101/102*	*Hatch.*	*Wild-Ang*	*Wild-Isl*	*Wild-SA*
Eggs-003	*	0.022	0.031	0.061*	0.020	-0.015	0.007	0.014	0.011	−0.006	0.002	0.026	0.055*	0.060*
Eggs-004	0.016*	*	−0.006	0.001	−0.002	0.007	−0.008	0.028	−0.024	−0.001	0.040*	0.011	0.006	0.005
Eggs-039	0.014*	0.016*	*	−0.009	0.010	0.017	0.017	0.036	−0.014	0.017	0.053*	0.009	0.004	−0.004
Eggs-043	0.011*	0.006	0.015*	*	0.025	0.037	0.039	0.064*	−0.005	0.046	0.090*	0.025	0.001	−0.014
Eggs-047	0.004	0.007	0.011*	−0.003	*	0.003	−0.001	−0.005	−0.007	−0.003	0.030*	−0.010	−0.006	0.006
Eggs-051	0.007	0.006	0.005	0.002	−0.002	*	−0.011	−0.009	−0.004	−0.011	−0.001	0.000	0.024	0.023
Eggs-055	0.011	0.024*	0.015*	0.010*	0.000	0.007	*	−0.008	−0.007	−0.011	0.004	−0.001	0.022	0.019
Eggs-059	0.003	−0.001	0.003	−0.001	−0.007	−0.008	0.009	*	0.013	−0.004	0.001	−0.010	0.023	0.028
Eggs-087	0.028*	0.021*	0.013*	−0.012	−0.021	−0.003	0.004	0.031*	*	−0.003	0.031	−0.004	−0.003	−0.005
Eggs-101/102	0.026*	0.013*	0.030*	0.009*	0.004	0.015*	0.029*	0.023*	0.021*	*	0.000	0.005	0.029	0.035
Hatchery	0.032*	0.023*	0.023*	0.017*	0.012*	0.018*	0.026*	0.017*	0.000	0.013*	*	0.026	0.070	0.0721
Wild-Ang	0.023*	0.020*	0.034*	0.006*	0.009*	0.016*	0.028*	0.020*	−0.006	0.008*	0.015*	*	−0.001	0.002
Wild-Isl	0.031*	0.023*	0.039*	0.017*	0.017*	0.022*	0.038*	0.023*	0.002	0.014*	0.013*	0.002	*	−0.015
Wild-SA	0.019*	0.016*	0.031*	0.012*	0.011*	0.018*	0.033*	0.019*	0.001*	0.007*	0.016*	0.002	0.000	*

Significant *p*-values are denoted by an asterisk.

1995) to calculate Nei's unbiased gene diversity (Nei 1987), number of alleles, allelic richness, inbreeding coefficients (F_{IS}), and observed heterozygosity. Allelic richness was calculated using the methods described by Petit et al. (1998). This method allows the number of alleles to be compared among populations independently of sample size (Leberg 2002). We tested for deviations from Hardy–Weinberg expectations using the modified exact test (Guo & Thompson 1992) for each locus and sampling locality, and we used FSTAT to conduct the global test for linkage disequilibrium among each pair of loci. For mtDNA, we tabulated the number of distinct haplotypes and Nei's (1987) gene diversity using MICROSATELLITE TOOLKIT version 3.1[1].

Hierarchical analysis of variance among egg samples

To examine whether genetic variance was attributable to differences among temporally spaced egg samples (Hypothesis One) F_{ST} (Weir & Cockerham 1984) was calculated using the software package ARLEQUIN (Schneider et al. 2000) for microsatellite data. For mtDNA (analyzed separately) a comparable statistic, $\phi_{ST,}$ was computed. This measure of genetic variance is interpreted similarly to F_{ST}, but differs by incorporating genetic distances among haplotypes in computation of sums-of-squares (Excoffier et al. 1992). ARLEQUIN was also employed to test the second hypothesis that wild-caught egg samples were representative of potential adult breeders obtained from wild and hatchery-reared sources. Hierarchical analysis of molecular variance (AMOVA) was used for both mitochondrial and microsatellites to partition genetic variance into components attributable to divergence between egg samples and Wild plus Hatchery adults (F_{CT}, ϕ_{CT}), and to divergence among the populations within these two groups (F_{SC}, ϕ_{CT}). A third hierarchical AMOVA that excluded Hatchery adults was also conducted to determine its contribution to the outcome of the preceding analyses. *p*-values for all statistics were generated by a bootstrapping procedure.

[1] (add-in for Microsoft EXCEL, written by S. Park, available at http://oscar.gen.tcd.ie/~sdepark/ms-toolkit).

Table 3. Mitochondrial ND4 haplotype frequencies among *H. amarus* eggs, wild adults collected in the Angostura (Ang), Isleta (Isl) and San Acacia (SA) reaches in 2003 and hatchery-reared adults.

Population	mtDNA-ND4 Haplotypes											
	A	*C*	*D*	*E*	*F*	*J*	*K*	*O*	*S*	*M*	*P*	*Q*
Eggs-003	0.6000	0.2750	0.0250	0.0250	0.0500	–	0.0250	–	–	–	–	–
Eggs-004	0.6923	0.0769		0.1154	0.0769	–	–	0.0385	–	–	–	–
Eggs-039	0.7179	0.0769	0.0513	0.0256	0.0256	–	0.1026	–	–	–	–	–
Eggs-043	0.7872	0.0638	0.0638	0.0213	0.0213	–	0.0213	0.0213	–	–	–	–
Eggs-047	0.6098	0.0976	0.1463	0.0976	0.0244	0.0244	–	–	–	–	–	–
Eggs-051	0.6154	0.2051	0.0769	–	0.1026	–	–	–	–	–	–	–
Eggs-055	0.5814	0.1163	0.0465	0.0465	0.1395	0.0465	–	0.0233	–	–	–	–
Eggs-059	0.5278	0.1389	0.1667	–	0.1111	0.0278	–	–	0.0278	–	–	–
Eggs-087	0.6750	0.1000	0.0500	0.0500	0.0500	–	0.0250	0.0500	–	–	–	–
Eggs-101/102	0.5581	0.1628	0.0465	0.0698	0.0698	–	0.0233	–	–	–	–	0.0698
Hatch.	0.4815	0.2222	0.0494	0.0123	0.1358	–	0.0494	–	–	0.0370	0.0123	–
Wild-Ang	0.6056	0.0845	0.1549	0.0141	0.0704	–	0.0282	0.0141	–	0.0282	–	–
Wild-Isl	0.7031	0.0488	0.1563	0.0469	0.0313	–	–	0.0156	–	–	–	–
Wild-SA	0.7500	0.0313	0.1250	0.0313	0.0625	–	–	–	–	–	–	–

Results

Microsatellites

Allelic richness (A_R) among egg samples was lowest at *Lco4* (4.880–8.532) and greatest at *Lco1* (18.688–23.896) (general diversity statistics are available from the authors on request). Allelic diversity was slightly greater in the hatchery-reared adult population compared to the wild adult population (11.215/9.706) (Table 1). Considering all loci, weighted average inbreeding coefficients (F_{IS}) across 10 egg samples ranged from −0.077 (Eggs-101/102) to 0.221 (Eggs-059). The value of F_{IS} for hatchery-reared adults was 0.401. Exact tests of linkage disequilibrium revealed no significant differences ($\alpha = 0.0033$) after Bonferroni correction (Rice 1989) for multiple comparisons. Significant departures from H–W expectations occurred in four out of 12 samples after Bonferroni correction ($\alpha = 0.00069$). In each case there was an excess of homozygotes.

AMOVA revealed that a significant proportion of genetic variation reflects differences among egg samples collected at different times ($F_{ST} = 0.0101$, $p < 0.0001$). A significant proportion of genetic variation was also attributable to differences among egg samples and the putative parental source group (Wild plus Hatchery) ($F_{CT} = 0.0103$, $p = 0.0034$) and to differences among samples within groups ($F_{SC} = 0.088$, $p < 0.0001$). Exclusion of Hatchery adults from this comparison did not alter the significance of this result ($F_{CT} = 0.0138$, $p = 0.0026$) and ($F_{SC} = 0.0066$, $p < 0.0001$). Of 91 possible pairwise comparisons among egg samples and putative parental stocks, 60 values of F_{ST} were significant at nominal $\alpha = 0.05$ (Table 2). Pairwise values of F_{ST} for wild adults across river reaches were not significant.

Mitochondrial DNA

We observed 12 ND4 haplotypes among the egg samples and the wild and hatchery-reared adult samples (n = 624) (Table 3). Haplotype diversity was lower for mtDNA than average gene diversity observed at microsatellite loci. Eleven haplotypes were detected in the egg and wild populations and eight haplotypes were found in the hatchery population including one rare haplotype (*P*) not seen in the other populations. Haplotype *A* was most common in all samples.

For mtDNA, ϕ_{ST} was not significant when only egg samples were considered ($\phi_{ST} = 0.0067$, $p = 0.1466$) and no significant genetic variation was attributable to differences among eggs and parental source populations ($\phi_{CT} = -0.0021$, $p = 0.6051$). Significant genetic variation was attributable to differences among populations within groups (eggs and wild/Hatchery) ($\phi_{SC} = 0.0182$, $p = 0.0039$). Removing the Hatchery sample resulted in non-significant within-group

variance (ϕ_{SC} = 0.0034; p = 0.2454), but significant among group variance (ϕ_{CT} = 0.0134; p = 0.0303). Only 12 of 91 pairwise values of ϕ_{ST} differed significantly from zero (α = 0.05), six of these involved Hatchery adults with wild adults and eggs (Table 2).

Discussion

Extirpation and extinction of four pelagic-spawning cyprinids (*Macrhybopsis aestivalis*, *Notropis jemezanus*, *N. simus simus*, *N. orca*) and dramatic decline in abundance of *H. amarus* have been attributed, in part, to the interplay of early life history and water management practices in the Rio Grande (Platania & Altenbach 1998). The existing fragmented and highly regulated Rio Grande negatively affects pelagic broadcast-spawners because eggs are transported downstream with currents, and dams preclude subsequent upstream movement. Alò & Turner (2004) demonstrated genetic effects of this process in *H. amarus*, namely, dramatic reduction of contemporary effective population size and a low N_e/N ratio. They examined several factors that could account for this observation. One possibility, temporal fluctuations in adult population size, was alone insufficient to account for the $N_e/N \approx$ of 0.001 observed in the largest extant wild adult population sampled between 1999 and 2001. The aim of our study was to evaluate the sweepstakes–mismatch alternative posed by Alò & Turner (2004) whereby *H. amarus* experiences high variance in reproductive success because of failures to match reproductive effort and environmental conditions.

Sweepstakes recruitment is proposed to affect variance in reproductive success in *H. amarus* as follows. Ecological and genetic data suggest that adult silvery minnow form local spawning aggregations prior to egg release (Alò & Turner 2004). Spawning aggregations represent a subset of the adult breeding population, and are slightly genetically divergent (within a reach) from one another as a result of the sampling process during their formation (Alò & Turner 2004). Progeny from these aggregations experience high but differential mortality, and the probability of recruitment success/failure is related to the location of the aggregation. For example, spawning just upstream of a diversion dam might result in total loss of annual production, whereas spawning farther upstream might favor egg retention and recruitment in the natal reach.

If differential survival among progeny is affecting variance in reproductive success and, in turn, influencing genetic diversity, then we would expect the following: (1) Eggs should maintain temporal-spatial genetic separation as they drift downstream into suitable (or unsuitable) nursery habitats. Sampling eggs at a fixed location over the spawning season should reveal genetic differences among temporally-spaced egg samples. (2) Egg samples should differ genetically from the adult spawning population as a result of being produced by relatively few adult breeders. Microsatellite DNA data collected on adult spawners and eggs confirm both of these predictions. These findings indicate that high variance in reproductive success may be responsible for the low ratio of N_e/N in *H. amarus*.

MtDNA data failed to reject the hypothesis that temporally-spaced egg samples differed genetically. It is likely that mtDNA had lower statistical power than microsatellites to detect genetic differences among samples (Li & Hedgecock 1998). Statistical power depends on samples sizes (which were roughly equal for the two datasets) and number of independent haplotypes (alleles) available for estimation of F-statistics (Waples 1989, Ruzzante et al. 1996). The mtDNA dataset had relatively few haplotypes with one haplotype (A) predominating in all samples whereas the number of microsatellite alleles was an order of magnitude higher. F-statistics from microsatellites are based on weighted averages across independent genetic loci, whereas mtDNA represents only a single locus. We opted to analyze microsatellite and mtDNA data separately to capitalize on differential inheritance patterns between the two marker classes. Maternal inheritance of the mtDNA genome permits insights into the female portion of the population, but in this case no striking differences were revealed between the two datasets.

Genetic diversity among eggs and parental stocks

Both microsatellite and mtDNA data revealed genetic differences between putative parental

stocks and their progeny. Differences were especially pronounced between egg samples and the Hatchery sample, with significant values of F_{ST} in all (except Eggs-087) pairwise comparisons (F_{ST} = 0.02, $p < 0.0001$, on average), and five out of 10 pairwise comparisons of ϕ_{ST} significant at nominal alpha levels (but not after Bonferroni correction for multiple tests). The small degree of divergence (F_{ST} = 0.0004) between the hatchery sample and the egg sample Egg-087 collected during the third spawning spike implies that hatchery reared fish may be spawning later than the majority of the wild population. Egg samples were also divergent from wild fish based on microsatellite but not mtDNA analysis. No significant genetic differences were observed among adults occupying different river reaches in the Rio Grande. In 2003, there was insufficient replication within reaches to test for the presence of genetically distinct spawning aggregations. Interestingly, the magnitude of F_{ST} among egg samples mirrors closely the value of F_{ST} (=0.008) reported among spawning aggregations in the San Acacia reach in 2000 (Alò & Turner 2004).

Microsatellite data indicated significant deviation from Hardy-Weinberg expected genotype frequencies, with an excess of homozygotes in four out of 10 egg samples. Eggs might experience a temporary Wahlund effect (Wahlund 1928) as progeny from different aggregations mix during downstream transport but such mixing is not sufficient to genetically homogenize egg samples. The Wahlund effect occurs when allele frequencies differ between cryptic subpopulations. When subpopulations mix there will be a deficiency of heterozygotes and an excess of homozygotes even if each subpopulation is in H-W equilibrium. The presence of null alleles may also explain the excess of homozygotes in some samples. In a simulation study we found that the major effect of null alleles was to inflate within group variances (i.e. F_{IS}) with minimal effect on among group variances (F_{ST}) (Moyer & Turner, unpublished). In several of the egg samples and in the wild adult population there was an excess of heterozygotes which suggests that a small number of breeding individuals produced these groups of eggs. When the number of breeding individuals is small, allelic frequencies will differ slightly between males and females because of binomial sampling error resulting in heterozygote excess (Robertson 1965, Rasmussen 1979, Falconer 1981). Egg sample 101/102 had the greatest excess of heterozygotes. The number of eggs collected during this spawning spike was very small compared to the peak spawning event (Figure 2) suggesting that far fewer individuals spawned.

A striking result was the inflated inbreeding coefficient (F_{IS} = 0.401) observed in the Hatchery sample, which was more than double the source population (Wild 2002 population F_{IS} = 0.158, unpublished data). Possible explanations for this result include differential survival of heterozygous and homozygous individuals between the hatchery and wild conditions. Passive hatchery environments can lead to relaxed selection and increased survival of less fit homozygotes that maybe selected against in the wild. However, we do not yet fully understand the genetic consequences of hatchery rearing and repatriation into the wild in *H. amarus*.

Sweepstakes–mismatch recruitment was originally postulated to explain low N_e/N estimates in many pelagic-spawning marine fishes and invertebrates (Hedgecock 1994, Ruzzante et al. 1996, Li & Hedgecock 1998, Turner et al. 1999). Our data indicate that this process can be extended to include freshwater fishes that like many marine organisms, depend on passive dispersion of reproductive products into an environment with patchily-distributed resources. Life-history traits that unite these two groups include a pelagic phase, high fecundity (maximum 5,000 eggs per female – S. P. Platania, pers. comm.) and Type III survivorship (i.e., precipitous mortality of early life stages and reduced mortality in adult stages) (Flowers et al. 2002). Unlike many marine fish and invertebrate species, *H. amarus* are short-lived (most individuals die following first reproduction at age one, Platania & Dudley, unpublished). Thus the effect on N_e /N should be more profound than in long-lived species that spread reproductive effort over several seasons, lowering lifetime variance in reproductive success among individuals (Gaggiotti & Vetter 1999).

This study underscores the importance of understanding the interplay of life history (especially early life history) and fragmentation in devising conservation plans for endangered aquatic organisms. Current strategies to preserve

genetic diversity (and maintain abundance levels) in *H. amarus* include collection of eggs from the wild and subsequent hatchery rearing, and development of a captive broodstock. Progeny from both sources are ultimately repatriated to the Rio Grande. Our data indicate that genetic variation will continue to be eroded by loss of production from adult breeders in the highly fragmented Rio Grande system. This effect will not be ameliorated in the hatchery as repatriated individuals will suffer similar losses of production. Furthermore, if individuals for broodstock are drawn from the wild, they too will suffer depletion of genetic diversity. Ultimately, a genetically diverse and sustainable population of *H. amarus* will depend on addressing the root causes of its decline. This would include reconnecting fragmented habitats and allocating sufficient water resources for survival and growth during critical early life-history stages.

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