

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

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Synopsis

A sweepstakes-mismatch process that results in large variance in reproductive success among individuals and spawning aggregations has been proposed to explain low genetic effective 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 hypothesized to account for very low N_e/N (≈ 0.001) observed in the Rio Grande silvery minnow, *Hybognathus amarus*. This species is a federally endangered freshwater fish that shares life-history features with marine pelagic spawners. We tested two key predictions of sweepstakes-mismatch hypothesis: (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. Eggs were sampled at prescribed time intervals at a fixed locality as they drifted downstream in river currents over the 2003 spawning season. Reproductive adults were sampled prior to spawning from wild and a hatchery-reared stock repatriated to the wild. Individual egg and adult samples were genotyped using seven microsatellite loci and a mitochondrial (mt) DNA locus. Significant genetic differences were revealed among egg samples, and egg samples were genetically divergent from the parental population. Both observations are consistent with the hypothesis that high variance in reproductive success among adult breeders is an important factor that lowers N_e/N in Rio Grande silvery minnow.

Introduction

Although remarkably tolerant of severe environmental conditions, freshwater fishes of the southwestern United States have suffered recent declines in abundance, local extirpation and extinction as human demand for water increases in this arid region (Minckley et al. 2003). River fragmentation by impoundment and large-scale diversion of water from rivers are implicated as major factors driving species decline and extinction throughout the Southwest (Minckley & Deacon 1968). The Rio Grande silvery minnow, *Hybognathus amarus*, 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 the Federal Republic of Mexico (Trevino-Robinson 1959) but now occupies only 5% of its historical range. The remnant population is restricted to a 280-km river reach in New Mexico that is fragmented by three water diversion dams (Angostura, Isleta, San Acacia) and two major storage dams (Cochiti, Elephant Butte) that were constructed between 1919 and 1975 (Bestgen & Platania 1991) (Figure 1).

Rio Grande silvery minnows produce pelagic eggs that are subject to drift downstream with river currents (Platania & Altenbach 1998). This early life-history feature is not commonly observed in river fishes. Most species produce demersal (sinking) and/or adhesive eggs, ostensibly as a strategy to retain eggs in upstream portions of a flowing stream or river. However, pelagic spawning was probably once adaptive in the turbid waters of the Rio Grande (Moore 1944) because demersal/adhesive eggs could quickly become covered by silt and fail to develop. In the historically free-flowing Rio Grande, downstream reaches most likely provided important resources for larval recruitment owing to increased productivity in these reaches (Vannote et al. 1980).

Pelagic early life-history has been blamed, in part, for the decline of the Rio Grande silvery minnow 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 (e.g. Elephant Butte Reservoir) (Platania & Altenbach 1998, Luttrell et al. 1999). Larvae that survive are prevented from migrating upstream as adults by diversion dams and other impoundments and may subsequently perish when downstream reaches dry in summer. Downstream transport and loss of egg production has had important consequences for abundance patterns and demography. These factors, along with dramatic reduction in geographic range size, led to listing as a federally endangered species in 1994 (United States Department of the Interior 1994).

A previous genetic study of the Rio Grande silvery minnow 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). Alò & Turner (2004) proposed that low contemporary N_e/N most likely results from high variance in reproductive success among individuals and spatially discrete spawning aggregations, caused by failure to match reproduction to appropriate resources for larval development and recruitment. This “sweepstakes-mismatch” process was initially proposed to explain low N_e/N in pelagic-spawning marine species (Hedgecock 1994, Li & Hedgecock 1998).

The sweepstakes-mismatch process may strongly affect N_e/N in Rio Grande silvery minnow through an interaction of pelagic life-history, large-scale water diversion, and habitat fragmentation by dams (Alò & Turner 2004). This interaction results in heavy, but differential

mortality of early life stages by entrainment through diversion dams and extensive river drying that often occurs in the summer months following spawning. In principle, the effect on N_e/N is maximal if groups of eggs spawned at a particular place and time represent production from a few breeders and that these are genetically distinct from other such groups of eggs. In this scenario, whole groups of closely related progeny are subject to severe, but differential mortality (i.e. family correlated survival – Waples 2002).

To evaluate the efficacy of the sweepstakes-mismatch hypothesis for explaining low N_e/N observed in Rio Grande silvery minnow, two null hypotheses were tested using genetic data: (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 eggs samples and prospective breeding adults. Rejection of these would imply that the sweepstakes-mismatch hypothesis is an important factor that lowers N_e/N in Rio Grande silvery minnow.

Materials and Methods

Sampling for Genetic analysis

Rio Grande silvery minnow eggs were collected at a single site, located approximately 16 kilometres 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 to 1 June 2003 and encompassed the entire spawning season (Figure 2). Spawning is apparently 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 the release of water from upstream reservoir storage to create an artificial flow spike with the intention of inducing a spawning response in Rio Grande silvery minnow. Prior to, and following the artificial release, there were two rainstorms that prompted two smaller spawning events (Figure 2). Eggs were obtained using modified Moore egg collectors (Altenbach et al. 2000). A total of 450 eggs were sub-sampled from the collection (approximately 45 eggs from ten, one-hour time blocks during three distinct bouts of spawning – Table 1, Figure 2).

Genetic samples of potential adult spawners were also taken for comparison to egg samples. Wild, reproductively capable adults (n=168) were collected from the three river reaches (Figure 1) (from north to south: Angostura, Isleta, and San Acacia) by seining, occasionally with the aid of a backpack electrofishing unit, between December 2002 and March 2003. Fishes were anesthetized in MS222 at the collection site and a small piece of caudal fin was removed and stored in 95% ethanol. Fishes were then placed in untreated river water to recover prior to release. Fin clips were also obtained from hatchery-reared Rio Grande silvery

minnow (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.

Genomic DNA was isolated from individual fertilized eggs by mechanically rupturing egg membranes and then suspending them in 25µl of sterile H₂O and from fin clips using the protocol outlined in Alò & Turner (2004). All samples were screened for genetic variation at seven variable microsatellite loci (*Lco1*, *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7* [Turner et al. 2004]), and *CA6* (Dimsoski et al. 2000). The following microsatellite loci were amplified in 10µl reactions using multiplex PCR: *Lco3*, *Lco4*, *Lco5* (1X PCR buffer, 2mM MgCl₂, 125µM dNTPs, 0.40µM each primer, 0.375U TAQ DNA polymerase), *Lco6*, *Lco7* (1X PCR buffer, 2.5mM MgCl₂, 125µM dNTPs, 0.40µM each primer, 0.375U TAQ polymerase), *Lco1*, *CA6* (1X PCR buffer, 2.5mM MgCl₂, 125µM dNTPs, 0.40µM each primer, 0.375U TAQ polymerase). PCR cycling conditions were: an initial denaturation cycle of 94°C for 2 min, followed by 30 cycles of 94°C 20sec, 50°C (*Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7*) or 52°C (*Lco1*, *CA6*) 20 sec, 72°C 30 sec. Microsatellite loci were amplified using fluorescein labeled primers and detected and scored using an ABI377 automated sequencer with Genescan software.

Genetic variation was also characterized in a 295 base pair (bp) fragment of the protein-encoding mitochondrial ND4 gene using single-strand conformational polymorphism (SSCP) analysis (Sunnucks et al. 2000) and nucleotide sequencing. PCR amplification and genetic characterization followed Alò & Turner (2004).

Data Analysis:

For each microsatellite locus and sampling locality, FSTAT Version 2.9.3.2 (Goudet 1995) was used to calculate Nei's unbiased gene diversity (Nei 1987), number of alleles, allelic richness, inbreeding coefficients (F_{IS}), and observed heterozygosity. Deviations from Hardy-Weinberg expectations were tested using the modified exact test (Guo & Thompson 1992) for each locus and sampling locality, and the global test for linkage disequilibrium was conducted for each pair of loci using FSTAT. For mtDNA, the number of distinct haplotypes and Nei's gene diversity were tabulated using Microsoft EXCEL.

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, ϕ_{ST} , was computed. This measure of genetic variance is interpreted similarly to F_{ST} , but differs in that it incorporates genetic distances among haplotypes in computation of sums-of-squares (Excoffier et al. 1992). ARLEQUIN (Schneider et al. 2000) 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 datasets 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. Finally, F_{ST} and ϕ_{ST} were

calculated among all sample pairs to further refine interpretation of genetic divergence patterns among all genetic samples. P-values for all statistics were generated by a bootstrapping procedure implemented in ARLEQUIN.

Results

Microsatellites

For the seven microsatellite loci considered the number of alleles ranged from a minimum of 11 at *Lco5* to a maximum of 47 at *Lco1* (Table 2). Allelic richness among egg samples was greatest at *Lco1* (18.688 - 23.896) and lowest at *Lco5* (2.813- 7.280). Wild and hatchery-reared adult populations had similar levels of allelic diversity, with the highest values observed at *Lco1* (23.078/22.837) and the lowest at *Lco5* (4.466/ 3.952) (Table 2). Considering all loci, weighted average inbreeding co-efficients (F_{IS}) across ten egg samples ranged from -0.019 (Eggs-047) to 0.203 (Eggs-059) (Table 3). The value of F_{IS} for hatchery-reared adults was 0.368, which exceeded that of wild-caught egg and adult samples (Table 3). Exact tests of linkage disequilibrium revealed no significant comparisons after Bonferroni correction for multiple comparisons. Significant departures from HW expectations were observed in five out of 14 samples examined (Table 3). In each case there was an excess of homozygotes.

AMOVA revealed that a significant proportion of genetic variation could be explained by differences among egg samples collected at different times ($F_{ST} = 0.0176$, $P < 0.001$).

Hierarchical analysis of molecular variance indicated that a significant proportion of genetic variation could be attributed to differences among egg samples and the putative parental source group (Wild plus Hatchery) ($F_{CT} = 0.0115$, $P = 0.0009$) and to differences among samples within groups ($F_{SC} = 0.0122$, $P < 0.0001$) (Table 4). Removal of Hatchery adults from this comparison

did not alter the significance of this result ($F_{CT} = 0.0146$, $P = 0.0028$) and ($F_{SC} = 0.0110$, $P < 0.0001$) (Table 4). Of 91 possible pairwise comparisons among egg samples and putative parental stocks, 74 values of F_{ST} were significant at nominal $\alpha = 0.05$ (Table 5). Pairwise values of F_{ST} for wild adults across river reaches were not significant (Table 5).

Mitochondrial DNA

Twelve mtDNA – ND4 haplotypes were observed in egg, wild and hatchery-reared adult samples (totaling 624 individuals). Haplotype diversity was lower for mtDNA than average gene diversity observed at microsatellite loci. A total of eleven haplotypes were detected in the egg and wild populations and eight haplotypes were observed in the hatchery population including one rare haplotype (P) not seen in the other populations. Haplotype A was most common in all samples (Table 6). Two haplotypes were present as singletons (P, S). Nucleotide sequences of all ND4 haplotypes are accessioned into the GenBank database under sequential accession numbers AY536873 to AY 536885 and AY 682043 to AY 682045.

For mtDNA, ϕ_{ST} was not significant when only the egg samples were considered ($\phi_{ST} = 0.0067$, $P = 0.1466$) (Table 7) indicating that haplotype frequencies do not differ among egg samples. No significant genetic variation was explained by differences among the eggs and the parental source populations ($\phi_{CT} = -0.0021$, $P = 0.6051$) (Table 4). 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 alters this result where within-group variance is not significant ($\phi_{SC} = 0.0034$; $P = 0.2454$), but among group variance is significant ($\phi_{CT} = 0.01344$; $P = 0.0303$). Only 12 of 91 pairwise values of ϕ_{ST} differed significantly from

zero ($\alpha = 0.05$), but eight significant values were identified in comparisons between Hatchery adults and wild adults and eggs (Table 7).

Discussion

Extirpation and/or extinction of four pelagic-spawning cyprinids (*Macrhybopsis aestivalis*, *Notropis jemezianus*, *N. simus simus*, *N. orca*) and dramatic decline in the abundance of Rio Grande silvery minnow have been attributed, in part, to the interplay of early life history and water management practices in the Rio Grande (Platania & Altenbach 1998). The fragmented and highly regulated system that now exists in the Rio Grande negatively affects pelagic broadcast spawners because eggs are transported downstream with currents and subsequent upstream movement is precluded by dams. Alò & Turner (2004) demonstrated genetic effects resulting from river fragmentation in Rio Grande silvery minnow, 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. The first of these, temporal fluctuations in adult population size, was alone insufficient to account for $N_e/N \approx 0.001$ observed in the largest extant wild adult population sampled between 1999 and 2001. The aim of the present study was to evaluate the efficacy of an alternative hypothesis posed by Alò and Turner (2004); that the Rio Grande silvery minnow experiences high variance in reproductive success due to the failure to match reproductive effort and environmental conditions for most matings – referred to as the sweepstakes-mismatch hypothesis.

Conceptually, sweepstakes recruitment is proposed to affect variance in reproductive success in the Rio Grande silvery minnow in the following way. 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 spawning 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 further upstream in slow-flowing water 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 to be true. First, 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. Second, 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 predictions. First, gene frequencies varied among temporally spaced egg samples, and secondly, frequencies differed between the egg samples and the putative parental population. Pairwise values of F_{ST} indicated that most samples differed genetically from one another. These findings provide evidence that high variance in reproductive success may be responsible for the low ratio of N_e/N that is observed in the Rio Grande silvery minnow.

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 the number of independent haplotypes (alleles) available for estimation of F -statistics (Waples 1989, Ruzzante et al. 1996). The mtDNA dataset was characterized by few haplotypes with one haplotype (A) predominating in all samples. Conversely, the number of independent alleles in the microsatellite dataset was an order of magnitude higher. F -statistics from microsatellites are based on weighted averages calculated over 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 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 ($P < 0.002$, on average) of F_{ST} observed for all pairwise comparisons, and five out of 10 pairwise comparisons of ϕ_{ST} significant at nominal alpha levels (but not after Bonferroni correction for multiple tests). 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 year 2000 (Alò & Turner 2004).

Microsatellite data indicated significant deviation from Hardy-Weinberg expected genotype frequencies, with an excess of homozygotes in five out of 10 egg samples as denoted by positive values of F_{IS} . It is possible that eggs experience a temporary Wahlund effect as progeny from different aggregations mix during downstream transport. Mixing is not sufficient, however, to homogenize egg samples genetically.

A particularly striking result was the highly inflated inbreeding coefficient ($F_{IS} = 0.368$) observed in the Hatchery sample. This is nearly double the source population (Wild 2002 population $F_{IS} = 0.223$, Turner et al. 2003). 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 a relaxation of selection which may result in increased survival of less fit homozygotes that are selected against in the wild. However, we do not yet fully understand the genetic consequences of hatchery rearing and repatriation into the wild in Rio Grande silvery minnow.

Conclusions

Sweepstakes-mismatch recruitment was originally postulated to explain low N_e/N observed in many pelagic-spawning marine fishes and invertebrates (Hedgecock 1994, Turner et al. 1999, Li & Hedgecock 1998, Ruzzante et al. 1996). Our data indicate that this process can be extended to include freshwater fishes that exhibit similar life-history traits and depend on passive dispersion of reproductive products into an environment with patchily-distributed essential resources. Life-history traits that unite these species 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, Rio Grande silvery minnow are short-lived (most individuals die following first reproduction at age one [Platania & Dudley, unpublished]) so the effect on N_e/N is expected to be more profound than in long-lived species that spread reproductive effort over several seasons, thus 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 Rio Grande silvery minnow include collection of eggs from the wild and subsequent hatchery rearing, and the 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 Rio Grande silvery minnow 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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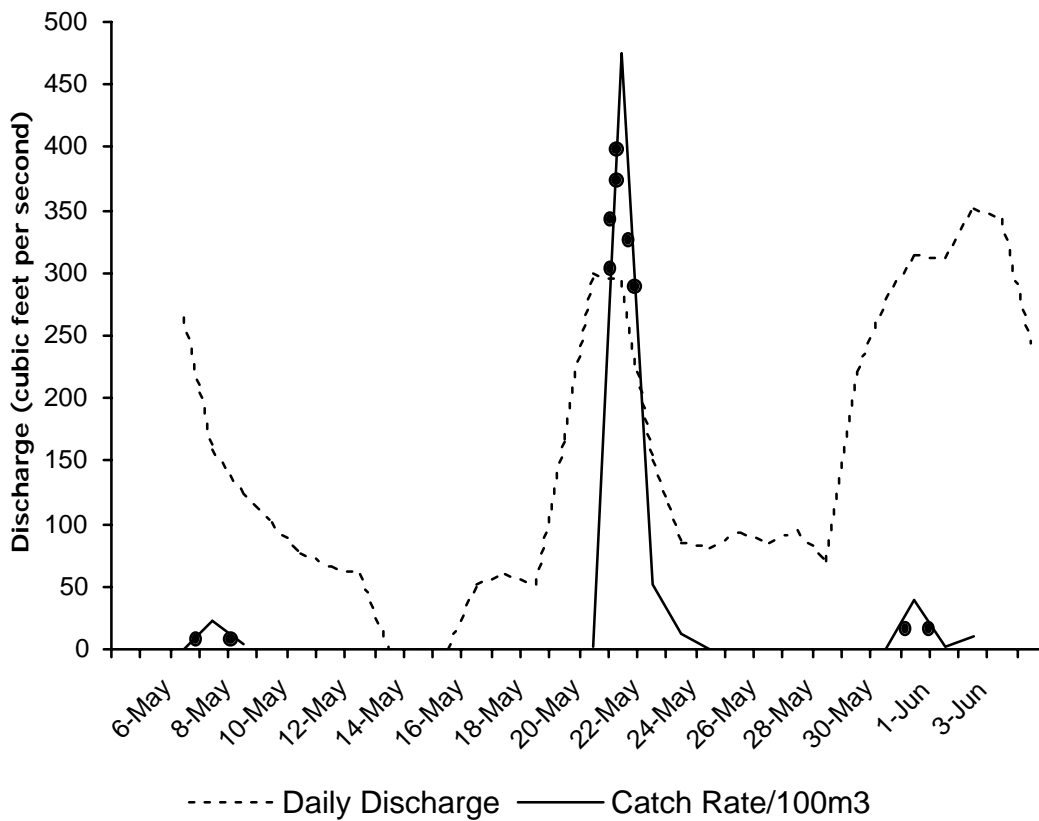


Figure 2: Mean daily discharge is indicated by a dashed line (cubic feet per second at USGS Guage 08358400). Rio Grande silvery minnow egg catch rate (per 100 m³ of water filtered) (Platania and Dudley 2004, unpublished) at the San Marcial egg collection site is shown by the solid line. Dots indicate the egg collections used for genetic analysis. (13th – 15th May 2003 daily discharge data is not available).

Table 1: Egg collection number (ACC2003-V:7- Museum Southwestern Biology, University of New Mexico), sample sizes (N), collection date, time of collection, minutes sampled and catch rate per 100 m³ of water filtered (Catch per unit effort of drifting eggs was calculated as the total number of eggs collected x volume of water sampled⁻¹ x 100).

	N	Collection Date	Time Start¹	Time Stop¹	Minutes Sampled¹	Catch Rate¹
Eggs-03-003	45	5/6/03	10.15	13.45	210	23.14
Eggs-03-004	46	5/7/03	10.30	14.30	240	5.16
Eggs-03-039	46	5/20/03	24.00	1.00	60	48.93
Eggs-03-043	51	5/20/03	9.00	10.00	60	962.90
Eggs-03-047	47	5/20/03	13.00	14.00	60	651.43
Eggs-03-051	45	5/20/03	17.00	18.00	60	227.15
Eggs-03-055	45	5/21/03	23.00	24.00	60	147.56
Eggs-03-059	37	5/21/03	8.30	9.30	60	94.17
Eggs-03-087	42	5/30/03	2.00	2.30	30	10.18
Eggs-03-101/102	46	6/1/03	11.15	1.15	240	12.81
Hatchery	81	Dec. 02	-	-	-	-
Wild 2003	168	Dec. 02-Mar. 03	-	-	-	-

¹ Data provided by S. P. Platania 2003 Rio Grande silvery minnow Spawning Periodicity Study (directed by Steven P. Platania; Museum of Southwestern Biology Accession ACC2003-V:7).

Table 2: Summary statistics for seven microsatellite loci for eggs samples and for putative parental source populations (Wild-Angostura, Isleta and San Acacia; Hatchery). Neis' unbiased gene diversity, observed number of alleles (total number of alleles across all populations), allelic richness (A_R) and average weighted inbreeding co-efficients (F_{IS}) are given for each locus and population.

Locus	Population Statistic	Eggs -003	Eggs -004	Eggs -039	Eggs -043	Eggs -047	Eggs -051	Eggs -055	Eggs -059	Eggs -087	Eggs- 101/102 Hatch.	Wild Ang	Wild Isl	Wild SA	
Lco1	Gene diversity	0.953	0.926	0.961	0.965	0.972	0.956	0.95	0.942	0.966	0.941	0.965	0.955	0.955	0.969
	Alleles (47)	21	19	28	26	29	24	23	22	22	25	34	29	30	27
	A_R	18.853	18.688	22.434	21.95	23.896	21.217	21.412	20.311	22	19.611	22.837	20.802	21.438	23.078
	F_{IS}	0.349	0.249	0.19	0.229	0.262	0.134	0.074	0.346	0.2	0.052	0.252	0.105	0.035	0.155
Lco3	Gene diversity	0.648	0.822	0.765	0.757	0.787	0.797	0.692	0.781	0.781	0.775	0.835	0.798	0.789	0.787
	Alleles (16)	7	8	10	8	9	9	7	9	7	9	12	9	7	10
	Richness	6.617	7.76	8.144	6.926	8.003	8.159	6.699	8.112	6.848	7.767	9.54	7.892	6.238	8.629
	F_{IS}	0.104	-0.022	-0.101	0.018	-0.01	-0.019	0.202	0.182	0.104	-0.168	0.369	-0.058	-0.072	-0.078
Lco4	Gene diversity	0.683	0.666	0.678	0.553	0.597	0.681	0.545	0.665	0.494	0.726	0.681	0.683	0.664	0.705
	Alleles (18)	6	5	9	6	8	9	6	6	7	10	11	7	6	6
	A_R	5.499	4.88	7.129	4.988	6.317	7.866	5.462	5.536	6.132	8.532	7.208	5.91	5.095	5.858
	F_{IS}	0.04	0.159	0.262	-0.052	0.055	-0.009	0.494	0.082	0.191	-0.12	0.603	-0.196	-0.321	-0.203
Lco5	Gene diversity	0.536	0.589	0.579	0.275	0.519	0.349	0.404	0.375	0.250	0.556	0.528	0.57	0.614	0.477
	Alleles (11)	5	8	5	5	3	3	3	3	5	6	5	4	5	3
	A_R	4.878	7.28	4.154	3.966	2.813	2.999	2.945	2.999	4.581	5.723	3.952	3.523	4.466	2.892
	F_{IS}	0.009	-0.358	-0.001	0.323	-0.631	-0.165	-0.28	-0.037	0.467	0.33	-0.007	-0.359	-0.318	-0.46
Lco6	Gene diversity	0.573	0.459	0.692	0.597	0.711	0.726	0.736	0.595	0.597	0.507	0.73	0.576	0.566	0.406
	Alleles (14)	8	6	9	8	12	10	8	8	7	9	12	10	10	8
	A_R	7.511	6	7.387	7.171	9.506	9.493	7.62	7.519	6.854	7.336	8.478	7.545	7.328	6.41
	F_{IS}	0.002	0.108	0.277	0.182	-0.148	-0.11	-0.056	0.1	0.196	-0.172	0.411	0.004	0.158	0.231
Lco7	Gene diversity	0.85	0.814	0.864	0.858	0.877	0.862	0.882	0.782	0.813	0.836	0.865	0.764	0.785	0.838
	Alleles (20)	11	7	14	12	12	9	11	7	11	10	13	11	12	11
	A_R	9.924	6.998	11.728	9.972	10.513	8.607	10.259	6.662	10.376	8.804	10.735	7.843	8.235	9.668
	F_{IS}	0.351	0.199	0.239	0.029	0.159	0.252	-0.05	0.275	0.114	-0.035	0.65	0.051	0.137	0.142
Ca6	Gene diversity	0.618	0.731	0.667	0.645	0.729	0.602	0.556	0.583	0.612	0.683	0.75	0.759	0.763	0.797
	Alleles (12)	6	7	8	7	8	7	6	7	7	7	11	9	10	7
	A_R	5.913	6.999	7.648	6.473	7.183	6.668	5.99	6.392	6.747	6.729	8.715	8.11	7.739	6.879
	F_{IS}	0.133	0.049	0.126	0.054	-0.161	0.141	-0.037	0.281	-0.046	-0.156	0.150	-0.179	-0.169	0.002

Table 3: Summary statistics for microsatellite and mtDNA – ND4 loci screened from Rio Grande silvery minnow eggs and adults. 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 22) and average weighted inbreeding co-efficient (F_{IS}) are give over all loci. For ND4 the gene diversity (h) and observed number of haplotypes are provided.

Population	Microsatellites						mtDNA-ND4		
	N	H_E	H_O	<i>Alleles/Locus</i>	<i>Richness</i>	F_{IS}	N	h	<i>Haplotypes</i>
Eggs-003	32	0.694	0.578	9.57	8.797	0.169	40	0.574	6
Eggs-004	25	0.735	0.697	9.00	8.788	0.053	26	0.514	5
Eggs-039	38	0.748	0.634	12.71	10.372	0.154	39	0.536	6
Eggs-043	44	0.669	0.605	11.14	9.331	0.097	47	0.378	7
Eggs-047	39	0.746	0.760	12.57	10.310	-0.019	41	0.601	6
Eggs-051	32	0.715	0.672	11.00	9.911	0.062	39	0.578	4
Eggs-055	29	0.684	0.644	9.71	9.107	0.061	43	0.639	7
Eggs-059	36	0.674	0.539	9.29	8.611	0.203	36	0.679	6
Eggs-087	43	0.725	0.751	12.00	9.943	-0.037	40	0.537	7
Eggs-101/102	32	0.645	0.548	10.29	9.787	0.152	40	0.660	7
Hatchery	81	0.778	0.492	15.29	10.804	0.368	81	0.703	8
Wild 2003	168	0.729	0.768	15.00	9.069	-0.054	152	0.495	7

Table 4: Hierarchical analysis of variance results and P-values among egg samples, between spawning spikes (eggs only) and among egg samples and adult Rio Grande silvery minnows. Standardized genetic variance attributable to differences among populations (F_{ST}), among groups (F_{CT}) and among populations within groups (F_{SC}).

	Eggs Only	P-value	Spikes	P-value	Eggs/Wild/Hatch	P-value	Eggs/Wild	P-value
<i>mtDNA-ND4</i>								
ϕ_{ST}	0.0067	0.1466	0.0042	0.1564	0.0162	0.0000	0.0171	0.0459
ϕ_{CT}	-	-	-0.0063	0.8690	-0.0021	0.6051	0.0134	0.0303
ϕ_{SC}	-	-	0.0104	0.0909	0.0182	0.0039	0.0037	0.2454
<i>μsats</i>								
F_{ST}	0.0176	0.0000	0.0178	0.0000	0.0236	0.0000	0.0254	0.0000
F_{CT}	-	-	0.0006	0.4204	0.0115	0.0009	0.0146	0.0028
F_{SC}	-	-	0.0172	0.0000	0.0122	0.0000	0.0110	0.0000

Table 5: Pairwise F_{ST} values (below diagonal) among egg samples, wild populations (Angostura [Ang], Isleta [Isl] and San Acacia [SA]) and the hatchery-reared adults (calculated from microsatellite data). Exact P-values are given above the diagonal. Significant F_{ST} values are shaded.

	Eggs -003	Eggs -004	Eggs -039	Eggs -043	Eggs -047	Eggs -051	Eggs -055	Eggs- 059	Eggs -087	Eggs -101	Hatch.	Wild -Ang	Wild -Isl	Wild -SA
Eggs-003	*	0.016	0.006	0.004	0.016	0.080	0.057	0.325	0.000	0.000	0.000	0.000	0.000	0.000
Eggs-004	0.016	*	0.021	0.003	0.124	0.009	0.003	0.110	0.000	0.001	0.000	0.000	0.000	0.008
Eggs-039	0.016	0.013	*	0.000	0.015	0.003	0.001	0.025	0.001	0.000	0.000	0.000	0.000	0.000
Eggs-043	0.015	0.024	0.033	*	0.000	0.319	0.002	0.506	0.993	0.000	0.000	0.000	0.000	0.000
Eggs-047	0.011	0.005	0.009	0.024	*	0.004	0.057	0.034	0.021	0.000	0.001	0.001	0.000	0.004
Eggs-051	0.008	0.015	0.016	0.002	0.013	*	0.073	0.956	0.678	0.001	0.000	0.000	0.000	0.000
Eggs-055	0.010	0.024	0.018	0.015	0.006	0.007	*	0.110	0.083	0.000	0.000	0.000	0.000	0.000
Eggs-059	0.004	0.009	0.014	0.000	0.008	-0.008	0.008	*	0.001	0.000	0.000	0.000	0.000	0.001
Eggs-087	0.032	0.039	0.033	-0.012	0.009	-0.002	0.010	0.029	*	0.000	0.008	0.000	0.000	0.005
Eggs-101	0.022	0.018	0.034	0.013	0.017	0.015	0.029	0.022	0.025	*	0.000	0.000	0.000	0.000
Hatchery	0.031	0.020	0.021	0.030	0.012	0.023	0.025	0.022	0.015	0.018	*	0.000	0.000	0.002
Wild-Ang	0.022	0.016	0.031	0.022	0.009	0.022	0.027	0.024	0.012	0.013	0.014	*	0.120	0.139
Wild-Isl	0.029	0.020	0.036	0.032	0.018	0.029	0.039	0.029	0.019	0.019	0.012	0.002	*	0.177
Wild-SA	0.018	0.013	0.028	0.024	0.011	0.022	0.030	0.022	0.015	0.013	0.014	0.002	0.002	*

Table 6: Mitochondrial ND4 haplotype frequencies among Rio Grande silvery minnow eggs (Eggs-003-Eggs-101/102) wild adults collected in the Angostura (Ang), Isleta (Isl) and San Acacia (SA) reaches in 2003 and hatchery-reared adults.

Population	<i>mtDNA-ND4 Haplotypes</i>											
	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
Hatchery	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	-	-	-	-	-	-	-

Table 7: Pairwise ϕ_{ST} -values (below diagonal) among egg samples, wild populations (Angostura [Ang], Isleta [Isl] and San Acacia [SA]) and the hatchery-reared adults (calculated from ND4 data). Exact P-values are given above the diagonal. Significant ϕ_{ST} values are shaded.

	Eggs -003	Eggs -004	Eggs -039	Eggs -043	Eggs -047	Eggs -051	Eggs -055	Eggs- 059	Eggs -087	Eggs -101	Hatch.	Wild -Ang	Wild -Isl	Wild -SA
Eggs-003	*	0.157	0.084	0.019	0.128	0.709	0.258	0.193	0.225	0.499	0.321	0.064	0.015	0.031
Eggs-004	0.022	*	0.494	0.365	0.406	0.279	0.537	0.108	0.923	0.382	0.047	0.210	0.271	0.313
Eggs-039	0.031	-0.006	*	0.625	0.221	0.164	0.130	0.058	0.766	0.135	0.011	0.208	0.288	0.452
Eggs-043	0.061	0.001	-0.009	*	0.091	0.053	0.034	0.014	0.498	0.023	0.001	0.058	0.331	0.724
Eggs-047	0.020	-0.002	0.010	0.025	*	0.329	0.400	0.489	0.568	0.469	0.039	0.719	0.534	0.278
Eggs-051	-0.015	0.007	0.017	0.037	0.003	*	0.655	0.580	0.452	0.659	0.391	0.370	0.091	0.140
Eggs-055	0.007	-0.008	0.017	0.039	-0.001	-0.011	*	0.585	0.561	0.749	0.280	0.384	0.083	0.139
Eggs-059	0.014	0.028	0.036	0.064	-0.005	-0.009	-0.008	*	0.189	0.464	0.365	0.681	0.093	0.105
Eggs-087	0.011	-0.024	-0.014	-0.005	-0.007	-0.004	-0.007	0.013	*	0.442	0.035	0.485	0.458	0.483
Eggs-101	-0.006	-0.001	0.017	0.046	-0.003	-0.011	-0.011	-0.004	-0.003	*	0.380	0.254	0.053	0.065
Hatch.	0.002	0.040	0.053	0.090	0.030	-0.001	0.004	0.001	0.031	0.000	*	0.026	0.001	0.004
Wild-Ang	0.026	0.011	0.009	0.025	-0.010	0.000	-0.001	-0.010	-0.004	0.005	0.026	*	0.412	0.318
Wild-Isl	0.055	0.006	0.004	0.001	-0.006	0.024	0.022	0.023	-0.003	0.029	0.070	-0.001	*	0.841
Wild-SA	0.060	0.005	-0.004	-0.014	0.006	0.023	0.019	0.028	-0.005	0.035	0.072	0.002	-0.015	*