

**Effects of habitat fragmentation on effective population size in  
the endangered Rio Grande silvery minnow**

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**Abstract:** *We assessed spatial and temporal patterns of genetic diversity to evaluate effects of river fragmentation on remnant populations of the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*). Analysis of microsatellite and mitochondrial (mt) DNA detected little spatial genetic structure over the current geographic range, consistent with high gene flow despite fragmentation by dams. However, maximum-likelihood analysis of temporal genetic data indicated that present-day effective population size ( $N_{eV}$ ) of the largest extant population of this species was 78 and the ratio of effective size to adult numbers ( $N_{eV}/N$ ) was  $\sim 0.001$  during the study period (1999 to 2001). Coalescent-based analytical methods provided an estimate of historical (river fragmentation was completed in 1975) effective size ( $N_{eI}$ ) that ranged between  $10^5$  and  $10^6$ . We propose that disparity between contemporary and historical estimates of  $N_e$  and low contemporary  $N_e/N$  result from recent changes in demography related to river fragmentation. Rio Grande silvery minnow produce pelagic eggs and larvae subject to downstream transport through diversion dams. This life-history feature results in heavy losses of yearly reproductive effort to emigration and mortality and extremely large variance in reproductive success among individuals and spawning localities. Interaction of pelagic early life history and river fragmentation has altered demographic and genetic dynamics of remnant populations and reduced  $N_e$  to critically low values over ecological time.*

## **Introduction**

Habitat destruction and fragmentation are arguably the most important general factors that drive global loss of species diversity (Vitousek et al. 1997; Young & Clark 2000). The direct demographic and genetic consequences of fragmentation for individual species and populations have been widely studied in terrestrial organisms through development of theory (e.g., Hanski 1999), manipulative experiments (e.g., Diffendorfer et al. 1995), and long-term demographic and genetic studies (e.g., Westemeier et al. 1998). Organisms restricted to lotic, freshwater habitats have received less attention than terrestrial organisms despite the fact that most rivers in the developed world are severely fragmented by dams and other structures that impede free movement of organisms through river corridors (Benke 1990; Dynesius & Nilsson 1994). There is a pressing need to understand the demographic and genetic consequences of river fragmentation for persistence of aquatic organisms (Dunham & Rieman 1999; Jager et al. 2001; Speirs & Gurney 2001).

We evaluated demographic and genetic effects of river fragmentation on the Rio Grande silvery minnow (*Hybognathus amarus*), a federally endangered fish species endemic to the Rio Grande Basin. Before 1970 this species was abundant and widely distributed from the Rio Grande headwaters in New Mexico to its confluence with the Gulf of Mexico (Fig. 1). The species is now restricted to a segment of the river bounded by two major reservoirs and fragmented by three dams (Bestgen & Platania 1991). Dams were designed primarily to divert water for agriculture and have been implicated in the decline and extirpation of at least four fish species in the Upper Rio Grande, including the Rio Grande silvery minnow (Platania 1991). All four species produce semibuoyant eggs

that are passively transported by river currents (Platania & Altenbach 1998). There is evidence that young fish fail to recruit to breeding populations because eggs and larvae are entrained through water diversion structures and are transported downstream into unsuitable nursery habitats (e.g., Elephant Butte Reservoir) (Platania & Altenbach 1998). Even if larvae survive to adulthood, dams block upstream return to natal sites or prevent recolonization in areas of local extirpation (Luttrell et al. 1999).

We focused on spatial and temporal patterns of abundance and genetic diversity of remaining wild populations of Rio Grande silvery minnow. The goals were to (i) provide baseline information about genetic diversity and abundance to help guide species recovery efforts and (ii) contribute to a more general understanding of the interplay of fragmentation, demography and life history, and genetic diversity for organisms in river ecosystems. We examined interactions of demography, life history, and genetic diversity explicitly by calculating the ratio of genetic effective population size ( $N_e$ ) to adult census size ( $N$ ). This ratio is expected to be unity in a Wright-Fisher idealized population, i.e., panmictic, 1:1 sex ratio, Poisson-distributed variance in reproductive success, and stable population size (Caballero 1994; Wang & Caballero 1999), but is expected to deviate from unity when idealized assumptions are violated, usually because of factors related to demography (e.g., fluctuating population size, mortality schedules) and life history (e.g., mating system, life span, variance in reproductive success) (Nunney & Elam 1994; Frankham 1995a). We estimated and interpreted  $N_e/N$  ratios in light of demographic and life history information gathered from intensive ecological monitoring of this endangered aquatic species.

There are a number of ways to estimate  $N_e$ , each with specific assumptions and biological interpretations (Waples 2002). We compared two distinct values of  $N_e$  for Rio Grande silvery minnow. First, we estimated variance effective size ( $N_{eV}$ ), which is commonly referred to as contemporary or current effective size (Waples 2002). Second, we estimated inbreeding effective size ( $N_{eI}$ ) with coalescent-based methods, which is referred to as the long-term or historical effective size because it is estimated over the time since common ancestry of all alleles in the population (Avice 2000; Garrigan et al. 2002; Waples 2002). We used current and long-term estimates of  $N_e$  in concert to gain contemporary and historical insight into the effects of river fragmentation on genetic diversity in Rio Grande silvery minnow.

## **Methods**

### **Study species**

The historical geographic range of the Rio Grande silvery minnow encompassed nearly the total length of the main stem Rio Grande and Pecos River in New Mexico and Texas, and museum records and ichthyological accounts indicate that the species was among the most abundant fishes in this region (Trevino-Robinson 1959). At present, it is restricted to the middle portion of the Rio Grande in New Mexico and occupies only about 5% of its historical range. Dramatic reduction in geographic range size and abundance led to the listing of this fish as federally endangered in 1994 (U.S. Department of the Interior 1994).

The Rio Grande silvery minnow is a small fish (maximum total length 13 cm) is mature at 1 year, is short lived (~90% die after first reproduction, S. Platania & R. Dudley, unpublished data), and has effectively nonoverlapping (i.e., discrete) generations. Despite small body size, they are highly fecund, with each female producing up to 5,000 (S. Platania, unpublished data). Fertilized eggs are about 1.0 mm in diameter when broadcast into the water column during spawning, but quickly swell with water to about 3.0 mm and become semibuoyant. Developing eggs and larvae drift passively with river currents for about 3 to 5 days (Platania & Altenbach 1998). Drift distances are relatively far (over 100 km) because spawning occurs during elevated river flows in springtime, usually in late April or early May.

### **Study site**

The middle Rio Grande, New Mexico, is a 280-km reach from Cochiti Dam to Elephant Butte Reservoir (Fig. 1). The channel is shallow, sandy, and braided, and the river is constrained by levees that limit extent and duration of seasonal flooding associated with springtime snowmelt runoff and precipitation. Average annual precipitation is low (<25 cm/year) and climatic conditions are semiarid to arid. 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: Cochiti, Angostura Isleta, and San Acacia (Fig. 1). We focused on the downstream-most reaches because the species is extremely rare in the Cochiti reach.

## Characterization of genetic diversity

From 1999 to 2001, adult Rio Grande silvery minnows were sampled at up to seven localities in the middle Rio Grande (Fig. 1). Each year, we collected fishes prior to spawning (December through March) by seining, occasionally with the aid of a backpack electrofishing unit. Fishes in 1999 and 2000 year classes were frozen whole, returned to the laboratory, and stored at -80°C. For the 2001 year class, captured fishes were temporarily anesthetized in MS-222 (Tricaine methanesulfonate 200mg/L river water) and released alive after a small portion of caudal fin had been removed from each individual and preserved in 95% EtOH. We deposited voucher and genetic materials in the Division of Fishes, Museum of Southwestern Biology (MSB) at the University of New Mexico (catalogue numbers MSB-49213, 49216, 49217, 49218, 49219, and 49221).

Total nucleic acids were isolated from air-dried fin clip samples with standard proteinase-K digestion and phenol/chloroform extractions. We screened DNA isolates for genetic variation at seven variable microsatellite loci (*Lco1*, *Lco3* – *Lco8*; Turner et al. 2004) and the protein-encoding mtDNA subunit-four gene (ND4).

Microsatellite loci were amplified using fluorescently labeled primers. We employed multiplex polymerase chain reaction (PCR) for the following combinations of microsatellite loci: *Lco3*, *Lco4*, *Lco5*, *Lco8* (1 µl 10 × PCR buffer, 2 mM MgCl<sub>2</sub>, 125 µM dNTPs, 0.25 µM each primer, 0.375U *Taq* DNA polymerase), and *Lco6*, *Lco7* (1 µl 10 × PCR buffer, 2.5 mM MgCl<sub>2</sub>, 200 mM dNTPs, 0.4 µM each primer, 0.375 U *Taq*). Locus *Lco1* was amplified singly (1 µl 10X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 125 µM dNTPs, 0.5 µM forward and reverse primers, 0.375 U *Taq*), with 94°C denaturation for 2 minutes, 25 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 52°C (*Lco1*), 50°C

(*Lco3*, *Lco4*, *Lco5*, *Lco8*), or 48°C (*Lco6*, *Lco7*), and 30 seconds extension at 72°C.

Resulting fragments were analyzed with Genescan Software designed for use with the ABI PRISM 377 DNA Sequencer (Applied Bioscience, Foster City, California).

We also amplified a 295 base pair fragment of the protein-encoding tDNA ND4 gene from each individual in 10 µl reactions that contained 1 µl template DNA, 1 µl 10× reaction buffer, 2 mM MgCl<sub>2</sub>, 125 µM dNTPs, 0.5 µM forward (5' - GAC CGT CTG CAA AAC CTT AA - 3') and reverse primer (5' - GGG GAT GAG AGT GGC TTC AA - 3'), and 0.375 U *Taq*. PCR conditions were 94°C initial denaturation for 2 minutes followed by 25 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds. Nucleotide sequence variation among individual fragments was visualized using single-strand conformational polymorphism (SSCP) analysis (Sunnucks et al. 2000), and representative haplotypes from each gel (~20%) were verified by direct sequencing using an ABI PRISM 377 DNA Sequencer.

Microsatellite allele and genotype frequencies, gene diversities (Nei 1987), inbreeding coefficients ( $F_{IS}$ ), and other summary statistics were tabulated with GENEPOP version 3.1d (Raymond & Rousset 1995) and ARLEQUIN (Schneider et al. 2000). Genetic samples were tested for departure from Hardy-Weinberg equilibrium expectations with exact tests (Guo & Thompson 1992), and linkage disequilibrium was tested across all pairs of loci with GENEPOP.

Population substructure was examined by computing Weir and Cockerham's (1984)  $F_{ST}$  for microsatellites and the analogous statistic,  $\Phi_{ST}$ , for mtDNA (Excoffier et al. 1992). Rivers are one-dimensional habitats, where gene flow may best be described by an isolation-by-distance model of gene flow (Wright 1943). We tested for genetic

isolation by distance with bivariate regression of pairwise genetic divergence on geographic distance (Rousset 1997). Microsatellite and mtDNA data were evaluated separately with ordinary least-squares regression, where the dependent variable was either values of  $F_{ST}/(1 - F_{ST})$  or  $\Phi_{ST}/(1 - \Phi_{ST})$  [mtDNA] calculated for all possible pairs of localities, and the independent variable was linear river distance (km). Degrees of freedom were adjusted to account for inflated Type I error rates associated with nonindependence (Hellberg 1994).

Spatial genetic structure among four localities sampled in the San Acacia reach in 2000 was evaluated using hierarchical analysis of molecular variance as implemented in ARLEQUIN. Microsatellite and mtDNA were analyzed separately, and weighted average  $F_{ST}$  and  $\Phi_{ST}$  were estimated. The null hypothesis that  $F_{ST}$  and  $\Phi_{ST}$  are equal to zero (i.e., no genetic structure among localities) was tested by resampling the original data set with replacement, assigning genotypes to four localities at random, while maintaining original sample sizes, and recalculating the test statistic. This procedure was repeated 10,000 times for each data set to generate null distributions of  $F_{ST}$  and  $\Phi_{ST}$ .

### **Estimating current $N_{eV}$ and $N_{eV}/N$**

We used the program MLNE to jointly estimate  $N_{eV}$  and the migration rate  $m$  from temporally spaced genetic samples via a pseudo-maximum-likelihood procedure (Wang and Whitlock 2003). The underlying model is conceptually similar to the well-known temporal method for estimating  $N_{eV}$  (Nei & Tajima 1981; Waples 1989). These previously developed models assume the study population is closed to migration, but

MLNE permits evaluation of  $N_{eV}$  in a focal population that receives migrants each generation from an infinitely large source population.

The MLNE analysis requires at least one pair of temporally-spaced samples obtained from the focal population and at least one genetic sample obtained from the source population taken at any time during the study. The focal population was from the San Acacia reach and the source population from the Angostura and Isleta reaches (Fig. 1). Between 1999 and 2001, the San Acacia reach harbored over 99% of remaining Rio Grande silvery minnow (R. Dudley & S. Platania, unpublished data). Samples from four localities were obtained in the San Acacia reach in 2000 and were pooled prior to analysis. Likewise, samples from Los Lunas and Angostura were pooled to comprise a single source population. In all analyses we assumed that genetic sampling did not change the available pool of reproductive individuals (Waples 1989, sampling plan I). For microsatellite DNA, the overall likelihood of  $N_{eV}$  is the product of the likelihoods of individual loci (Wang 2001). The ratio  $N_{eV}/N$  was computed by dividing the estimate of  $N_{eV}$  by the harmonic mean of midpoint values of  $N$  computed across 1999, 2000, and 2001 generations (Appendix).

MtDNA data were analyzed separately using MLNE and likelihood scores are evaluated for a haploid locus (Wang 2001). In this case,  $N_{eVf}$  is variance effective size for the female portion of the population estimated over the sample period. Likewise,  $m_f$  represents the fraction of female migrants from source to focal populations.

### Estimating long-term $N_{ef}$

We used an analytical method based on coalescent theory of gene genealogies to estimate the parameter  $\Theta$ , as implemented in the computer program Fluctuate (Kuhner et al. 1998).

For a neutrally evolving, haploid locus in a panmictic population of stable size,

$$\Theta = 2N_{ef}\mu, \quad (1)$$

where  $N_{ef}$  is the female inbreeding genetic effective population size and  $\mu$  is the per-site mutation rate (Kingman 1982, Ballard and Whitlock 2004). Inbreeding effective size is the appropriate measure of  $N_e$  because we are tracking the probability that two randomly chosen haplotypes from our sample were identical by descent (i.e., coalesced) in the previous generation, and then continuing backwards in time to the point where all haplotypes shared common ancestry (Orive 1993). Fluctuate runs were set as follows: searches were initiated with a starting value of  $\Theta$  based on Watterson's (1975) estimator; nucleotide frequencies were set to observed values; transition/transversion ratio was set to a value of two; and searches included 100 short chains with 10000 steps each and 10 long chains with 100000 steps each. Three separate Fluctuate runs, each with different random number seeds, were examined to confirm that estimates of  $\Theta$  and 95% confidence intervals (CIs) were stable and consistent among runs. Fluctuate also allows relaxation of the stable population size assumption generally required for estimation of  $\Theta$  and permits simultaneous estimation of  $\Theta$  and rate of population growth (or decline) scaled in terms of the number of mutations,  $g$ . A fourth Fluctuate run with the same initial settings as above was performed to evaluate  $\Theta_g$  and  $g$ . The genealogical approach we employed was computationally intensive, thus, our analysis focused only on the mtDNA ND4 fragment data pooled across localities and year classes.

We substituted estimates of  $\Theta$  from Fluctuate and three estimates of  $\mu$  obtained from the literature into Eq. 1 and solved for  $N_{\text{ef}}$ . Provided that sex-ratio is unity,  $N_{\text{ef}}$  for the entire population (male + female) is equal to  $2N_{\text{ef}}$  (Ballard and Whitlock 2004).

## Results

### Characterization of genetic diversity

Amplification of seven microsatellite loci yielded clearly resolvable and consistent PCR products amenable to scoring with GENESCAN software. The number of distinct alleles identified at each locus ranged from eight to 46 when tabulated across all localities (Table 1). Statistical testing revealed significant departures from Hardy-Weinberg equilibrium for three of seven loci (*Lco1*, *Lco4*, *Lco8*), with an excess of homozygotes observed in each case. The weighted average inbreeding coefficient ( $F_{IS}$ ) over all loci was 0.129. There was no evidence of linkage disequilibrium at nominal  $\alpha = 0.05$  among loci when compared in pair-wise fashion across all loci and Bonferroni corrected for multiple tests.

Nucleotide sequencing and SSCP characterization of the ND4 region of mtDNA yielded 13 distinct haplotypes from a total of 249 adult individuals sampled (Table 1). Haplotype diversity was lower than average gene diversity observed for microsatellites (Table 1). The most common haplotype (A) was present in 75% of individuals. Two haplotypes (D and F) were present at a frequency  $> 5\%$ , and remaining haplotypes occurred either at low frequency ( $< 5\%$ ) or were singletons. Nucleotide sequences are deposited in Genbank under sequential accession numbers AY536873-AY536885.

Regression analysis indicated little evidence for an isolation-by-distance model of gene flow. Slopes of regression of genetic distance on geographic distance were not

significantly different from zero considering microsatellites (slope =  $1.0 \times 10^{-5}$ ;  $p = 0.58$ ) and mtDNA data (slope =  $2.8 \times 10^{-5}$ ;  $p = 0.92$ ), respectively. Significant genetic structure was detected among localities in the San Acacia reach collected in 2000 for microsatellite ( $F_{ST} = 0.008$ ,  $p = 0.001$ ) and mtDNA data ( $\Phi_{ST} = 0.072$ ,  $p = 0.005$ ). Examination of the mtDNA-SSCP data indicated that  $\Phi_{ST}$  was largely attributable to the absence of haplotype D in the Former LFCC confluence locality.

### **Current effective size, $N_{eV}$**

Estimates of  $N_{eV}$  for the San Acacia focal population ranged from 42 to 93 for microsatellites (Table 2). For mtDNA,  $N_{eVf}$  ranged from 11 to 26 (Table 2). Estimates of  $N_{eVf}$  are slightly smaller than one-half the values of  $N_{eV}$  in all cases, which is the expected difference of  $N_{eV}$  estimated from diploid and haploid gene markers in an idealized population. Migration rates from source populations (Angostura and Los Lunas) to the focal San Acacia population were  $m \geq 0.8$  for microsatellites and  $m_f \geq 0.1$  for mtDNA (Table 2). In general, 95% CIs were broader for parameter estimates based on mtDNA than those based on microsatellites because fewer independent loci and alleles were available to estimate parameters of interest (Waples 1989).

Considering all temporal contrasts combined,  $N_{eV}$  was 78, and the ratio of effective size to adult population size ( $N_{eV}/N$ ) ranged from 0.0003 to 0.0530 depending on how harmonic mean  $N$  (= adult population number) was calculated (Appendix). The lower value was based on  $N$  equal to the product of mean density, reach length (in meters), and average channel width in the San Acacia reach (181 m), and is probably an overestimate of  $N$  and an underestimate of  $N_{eV}/N$ . The higher value was based on  $N$  equal

to the product of mean density and river length, and is probably an underestimate of  $N$ , and thus an overestimate of  $N_{eV}/N$ . A value of  $N_{eV}/N = 0.001$  was obtained when harmonic mean  $N$  was estimated as the product of mean density from population monitoring data, reach length, and width = 50 m (which is mean channel width minus 1 SD).

### **Long-term effective size, $N_{eL}$**

Coalescent-based, genealogical analyses of mtDNA ND4 fragments indicated that  $\Theta = 0.0091$  when averaged across three independent Fluctuate runs (Table 3). When population growth or decline were included in the model, the value of  $\Theta_g$  was larger than  $\Theta$ , and  $g$  was positive (Table 3), which indicated a growing population. Substitution of  $\Theta$ ,  $\Theta_g$ , and three values of  $\mu$  into the Eq. 1, solving for  $N_{eff}$ , and then multiplying by two yielded long-term  $N_{eL}$  that ranged between  $8.77 \times 10^5$  and  $6.56 \times 10^6$  (Table 4).

### **Discussion**

The task of identifying specific factors that affect genetic diversity of imperiled populations is often complicated by incomplete understanding of ecological parameters such as population numbers, age structure, migration dynamics, and lack of baseline genetic data that precedes major disturbance and fragmentation events. Our investigation of the endangered Rio Grande silvery minnow addressed these problems by (i) documenting spatial and temporal patterns of genetic diversity over three generations in its present geographic range, (ii) evaluating the relationship of spatial and temporal genetic changes to changes in adult numbers over three generations, and (iii) estimating

effective population size with computational methods that reflect current and historical population dynamics, respectively. In the case of Rio Grande silvery minnow, the combination of ecological and temporal genetic information is essential to infer the nature and magnitude of processes that shaped genetic diversity in the past, and at present, in this endangered fish species.

### **Spatial and temporal genetic diversity, $N_{eV}$ and $N_{eV}/N$**

Population genetic theory indicates that critical losses of genetic diversity and concomitant accumulations of deleterious alleles are not generally expected over ecological time unless  $N_e \leq 100$  (Luikart et al. 1998; Lynch & Gabriel 1990). The maximum-likelihood estimate of  $N_{eV}$  for the San Acacia population of Rio Grande silvery minnow was 78 and is two to four orders of magnitude lower than harmonic mean  $N$  for this reach, depending on the sampling interval and assumptions for calculating  $N$ .

The ratio  $N_e/N$  has received recent attention in conservation biology because its interpretation explicitly links demographic (adult census size, mating system, life span, etc.) and genetic dynamics of a population (Nunney and Elam 1994). In an idealized population the ratio  $N_e/N$  is one, but idealized conditions are rare in natural populations. Empirical and theoretical examinations of  $N_e/N$  suggest that this number usually takes values between 0.25 – 0.5 under a variety of life histories, mating systems, and demographic scenarios (Nunney & Elam 1994; Frankham 1995a). However, Nunney and Elam's (1994) theoretical work assumes that individuals in each age class have an equal probability of surviving to the next age class (i.e., Type II survivorship), which does not hold for most fishes.

Of the potential factors that theoretically lower  $N_{eV}/N$  below idealized expectations, two appear plausible for Rio Grande silvery minnow. First, temporal fluctuations in adult census size, most notably an order of magnitude decrease in abundance in 2001 (Appendix), could lower  $N_{eV}/N$ . However, if census size equals  $10^5$  for generation one and  $10^4$  for generation two (see Appendix), then adult census size of 24 individuals is required in generation three to account for  $N_{eV} = 78$ , assuming  $N_{eV}$  is related to harmonic mean  $N$  in an otherwise idealized population. Ecological monitoring data suggest that this is an unrealistically small number of adult fishes and that fluctuation of  $N$  alone cannot account for low  $N_{eV}/N$ .

Population structure can also affect  $N_{eV}/N$ . A small, but significant proportion of genetic variance was attributable to differences among San Acacia reach localities collected in 2000. Spatial genetic variance may represent formation of spatially discrete spawning aggregations, each consisting of a subset of the total number of breeding individuals in the reach. If local aggregations were constant in size and stable through time, then population structure would be expected to increase  $N_{eV}/N$  minimally about 0.8% and maximally about 7% (found by substituting  $F_{ST}$  and  $\Phi_{ST}$  into Eq. 3 of Waples [2002]) relative to the result based on pooling localities prior to analysis (i.e., assuming no structure). Genetic sampling and ecological monitoring data suggest that these aggregations are not demographically stable, that is, local abundance varies widely across localities and from year to year despite similar sampling effort. In theory, when variance in productivity among localities exceeds idealized expectation,  $N_e$  is always lowered relative to  $N$  even when individuals assort randomly into temporary “subpopulations” each breeding season (Whitlock & Barton 1997).

We propose that variance in productivity among spawning localities and variance in reproductive success among individuals is likely to lower  $N_{eV}/N$  below theoretical expectation in Rio Grande silvery minnow. These factors have been posited to explain low  $N_e/N$  in species characterized by Type III survivorship curves (Hedgecock 1994; Turner et al. 2002). Type III survivorship is found in species with enormous capacity for reproduction, low allocation of energy per individual offspring, and extremely high mortality in early life stages. Such species can be subject to a "sweepstakes-mismatch" process whereby genetically related groups of eggs and larvae experience high but differential mortality as they disperse into highly heterogeneous environments (Hedgecock 1994). Sweepstakes recruitment dynamics are hypothesized to produce large variances in reproductive success far in excess of idealized expectation, ultimately yielding low  $N_e/N$  (Hedgecock 1994).

The sweepstakes-mismatch process is potentially strongly enhanced by river fragmentation in Rio Grande silvery minnow. Empirical studies of drifting particles that mimic Rio Grande silvery minnow eggs in size and density suggest that a very small fraction of eggs are retained in any river reach and that the majority of production is swept to the most downstream portion of the system at Elephant Butte Reservoir (Dudley 2004). The probability that offspring recruit from a particular spawning aggregation or individual breeding pair may depend on where spawning occurs. For example, spawning just upstream of a diversion dam might result in total loss of production, whereas spawning further upstream in slow-flowing water might favor egg retention and recruitment in the natal reach.

Estimation of  $N_{eV}$  focused on the San Acacia reach and inferences above are limited to that population. However, it is likely that upstream populations in the Angostura and Isleta reaches experience similar, if not exaggerated, effects on  $N_{eV}/N$  due to relatively short distances between diversion dams and narrower, more channelized geomorphology (P. Tashjian, personal communication) that increases egg and larval drift rates (Dudley 2004). One potentially complicating factor of the current species distribution is that the Wang and Whitlock (2003) model used to estimate  $N_{eV}$  assumes migration from an infinite source population. Wang and Whitlock (2003) clearly demonstrate, however, that their model is robust to violations of this assumption.

Three of seven microsatellite loci we examined were out of Hardy-Weinberg equilibrium, most likely because one or more null alleles were present in these loci. Null alleles can potentially influence estimation of  $N_{eV}$ . We argue that effects of null alleles on our analysis and interpretation are minimal for two reasons: (i) individual microsatellite loci produced very similar estimates of  $N_{eV}$  (using the generalized temporal-method estimator of Waples 1989, data not shown) and (ii)  $N_{eVf}$  based on MLNE analysis of mtDNA was very close to its expected value given that  $N_{eV}$  was correct (Table 2).

Finally, our interpretation of  $N_{eV}/N$  depends on very rough estimation of adult numbers,  $N$ . Chapman (1990) discussed some of the problems associated with attempting to relate catch-per-unit-effort (CPUE) values to species abundance or density, and we emphatically concur with those conclusions. Our estimates of adult numbers are based on standardization of monitoring data and a number of critical assumptions about fish distribution in the Rio Grande. This estimation procedure was intended to provide a

range of plausible values for  $N$ . Note that observed  $N_{eV}/N$  is lower than expected for an idealized population and lower than empirically and theoretically derived estimates of  $N_e/N \approx 0.1 - 0.5$ , regardless of estimates of  $N$  used.

### **Comparison of current $N_{eV}$ and long-term $N_{eI}$**

An important element of this study was the comparison of current and long-term estimates of  $N_e$  in an attempt to understand whether genetic diversity (in terms of  $N_e$ ) was higher prior to river fragmentation completed about 30 silvery minnow generations ago. Others have accomplished this by comparing genetic diversity of specimens obtained prior to disturbance with present-day populations (Bouzat et al. 1998). This approach could not be implemented in Rio Grande silvery minnow because all specimens obtained before our study are fixed in formalin, which precludes reliable DNA isolation and characterization.

Our approach relies on the fact that  $N_{eV}$  and  $N_{eI}$  are equal in an idealized population (Whitlock and Barton 1997), but differ substantially under non-idealized conditions (Crow & Denniston 1988). For example,  $N_{eV}$  is sensitive to population size fluctuations over the sampling interval and can be used to detect recent population bottlenecks (Luikart et al. 1998). Long-term estimates of  $N_{eI}$  are not as sensitive to recent changes, but rather record both historical and contemporary changes in population size (Avice 2000).

If life history and river fragmentation interact to drive  $N_{eV}/N$  to low values in contemporary Rio Grande silvery minnow populations, then we would predict that effective size prior to fragmentation was much larger. The coalescent-based estimate of

historical  $N_{eI}$  is between  $10^5$  and  $10^6$ . It is not possible to estimate historical  $N_{eI}/N$  directly because we are unsure of pre-fragmentation adult census size, although it was probably large (Trevino-Robinson 1959). However, if  $10^4 - 10^5$  adult fishes occupy 5% of the historical range, then we can estimate the historical census size to be several million fishes. This would put the long-term  $N_e/N$  ratio of Rio Grande silvery minnow in the neighborhood of empirically-derived estimates of  $N_e/N \approx 0.1 - 0.5$  for other wild organisms (Frankham 1995a).

Caution is warranted in our interpretation of long-term  $N_{eI}$ . Waples (2002) pointed out that variances of long-term  $N_e$  may be large because of uncertainty surrounding estimation of  $\mu$ , and the effects of selection, historical fluctuations of  $N$ , and other processes that cannot be known or estimated with much confidence. We attempted to deal with uncertainty in  $\mu$  by using published estimates obtained from fishes and mammals. Note that an order of magnitude increase in  $\mu$  will result in an order of magnitude decrease in  $N_{eI}$ .

Two observations from coalescent analysis, namely that  $\Theta_g > \Theta$  and  $g$  was a positive number, suggested that the Rio Grande silvery minnow population was growing exponentially despite recent declines in abundance. Genetic patterns consistent with exponential growth are commonly observed in rapidly declining species (Lavery et al. 1996; Garrigan et al. 2002) because of influence of past events (e.g., population growth preceding rapid decline) on coalescent-based estimates of  $\Theta$ . Our conclusion that  $N_{eI} \gg N_{eV}$  is supported under all scenarios we examined. The most likely explanation for this difference is a recent decline in effective size that coincides with river fragmentation.

## **Implications for management**

Low current  $N_e/N$  has important implications for management of this species. Generation time in the species is short (1 year) and so concomitant reduction in  $N_e$  from poor recruitment cannot be compensated by future reproduction. In years when recruitment success is high, harmonic  $N_e$  is expected to remain low because of inevitable recruitment variation among years. As a consequence, Rio Grande silvery minnow populations should experience losses of allelic diversity and heterozygosity in subsequent generations due to low effective size ( $N_e < 100$ ), despite relatively large adult numbers. In addition, we expect mean relatedness among individuals to increase, which will lead directly to inbreeding in future generations.

Secondly, if fragmentation remains unabated then large numbers of adult fishes must be maintained (e.g., through hatchery supplementation) in the wild to meet generally prescribed levels of genetic diversity. For example, if we assume  $N_e/N \sim 0.001$ , more than 500,000 adult fishes are required to maintain  $N_e = 500$ , the value at which 95% of neutral genetic variation is maintained over evolutionary time scales (Frankham 1995b). Over 5 million adult fishes must be maintained in the wild to approach  $N_e = 5000$ , a theoretical value for which sufficient levels of quantitative genetic variation are maintained over evolutionary time (Lande 1995). If recent river fragmentation is the primary cause of high variance in productivity and reproductive success, then ameliorating or removing effects of river fragmentation is probably the best way to ensure long-term persistence of the species and diminish the need for hatchery supplementation.

A hatchery program for Rio Grande silvery minnow was implemented by the U.S. Fish and Wildlife Service in December 2001, and more than 200,000 marked fishes have been released into the Angostura reach since that time. These individuals ostensibly began recruiting to the adult spawning population in spring 2002. Genetic data we collected provide a baseline from which to evaluate the effects of hatchery rearing and supplementation on the dynamics of genetic diversity of the species. One important finding was evidence for extensive gene flow among localities in the wild despite recent river fragmentation. This suggests it is not necessary to design a hatchery breeding plan to maintain spatial patterns of genetic diversity. Rather, breeding efforts should aim to maximize global  $N_e$  in Rio Grande silvery minnow.

## **Conclusions**

Conservation biologists sometimes distinguish between demographic and genetic processes and their effects on the likelihood of persistence of an endangered species. Lande (1988) argues that the time scale over which stochastic demographic processes can lead to extinction are generally much shorter than time scales required for extinction by genetic causes (e.g., accumulation of deleterious mutations, loss of fitness due to inbreeding) and thus, should receive highest priority for management. An important, but sometimes overlooked point Lande raised was emphasis on interactions of demographic and genetic factors in extinction. Our results show that genetic effective size in the Rio Grande silvery minnow is much smaller than expected based on adult census size, in fact, it is sufficiently small to warrant concern about extinction from genetic factors in the long term (Higgins and Lynch 2001). Underlying ecological factors that lower effective

population size likely occurred relatively recently and are related to demographic effects caused by an interaction of life history and extensive river fragmentation. Further refinement of our understanding of demography, life history, and genetic diversity and their interactions will ultimately lead to deeper insight into the processes of biological extinction and appropriate conservation steps to prevent it (Westemeier et al. 1998; Soulé & Mills 1998, Young & Clark 2000).

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**Table 1. Summary statistics for microsatellite and mtDNA data obtained from Rio Grande silvery minnow, tabulated by collection locality (Fig. 1) and year obtained.**

<i>Sampling locality</i>	<i>Year obtained</i>	<i>Microsatellites<sup>a</sup></i>				<i>MtDNA<sup>b</sup></i>		
		<i>n</i>	<i>H<sub>E</sub></i>	<i>H<sub>O</sub></i>	<i>Mean alleles/locus</i>	<i>n</i>	<i>h</i>	<i>haplotypes</i>
San Marcial	1999	46	0.688	0.622	9.3	42	0.374	5
former LFCC confluence	2000	29	0.752	0.658	10.9	33	0.119	3
San Marcial	2000	60	0.734	0.689	13.0	45	0.351	4
Bosque del Apache	2000	58	0.684	0.527	10.7	8	0.536	2
San Antonio	2000	47	0.743	0.659	11.6	29	0.589	5
San Acacia	2001	64	0.699	0.623	12.1	26	0.412	6
Los Lunas	2001	64	0.689	0.542	11.7	42	0.489	9
Angostura	2001	29	0.744	0.720	9.4	24	0.667	5
Averaged across localities		49.6	0.716	0.628	11.09	31.1	0.442	4.88

<sup>a</sup>Abbreviations: n, sample size; H<sub>E</sub>, Hardy-Weinberg expected heterozygosities; H<sub>O</sub>, direct count heterozygosities.

<sup>b</sup>Abbreviations: n, sample size; h, Nei's (1987) nucleon diversity; haplotypes, total number of distinct mtDNA haplotypes.

**Table 2. Pseudo-maximum likelihood estimates of variance effective size ( $N_{eV}$ ), female variance effective size ( $N_{eVf}$ ), migration rate ( $m$ ), and female migration rate ( $m_f$ ) across each pair of year classes, and for all year classes combined (contrast).**

<i>Contrast</i>	<i>Parameter*</i>	<i>ML estimate</i>	<i>-95% CI</i>	<i>+95%CI</i>
1999 vs. 2000	$N_{eV}$	92	70	133
	$N_{eVf}$	26	10	119
	$m$	0.87	0.72	0.98
	$m_f$	0.10	0.00	0.67
1999 vs. 2001	$N_{eV}$	42	28	70
	$N_{eVf}$	11	5	$\infty$
	$m$	0.80	0.52	1.00
	$m_f$	0.54	0.00	1.00
2000 vs. 2001	$N_{eV}$	93	66	157
	$N_{eVf}$	17	9	101
	$m$	0.56	0.30	0.86
	$m_f$	0.38	0.00	1.00
All combined	$N_{eV}$	78	62	102
	$N_{eVf}$	22	10	241
	$m$	0.86	0.70	0.98
	$m_f$	0.17	0.00	0.70

\*Parameter estimates reflect contemporaneous estimates for the focal population of Rio Grande silvery minnow located in the San Acacia reach of the Middle Rio Grande sampled from 1999 to 2001.

**Table 3. Estimates of  $\Theta$  from coalescent-based analysis calculated with the program Fluctuate (Kuhner et al. 1998).**

Parameter*	Estimate	-95% CI	+95%CI
$\Theta_1$	0.0095	0.0076	0.0120
$\Theta_2$	0.0091	0.0070	0.0126
$\Theta_3$	0.0088	0.0066	0.0117
$\Theta_g$	0.0162	0.0147	0.0177
$g$	1046.2	803.5	1289.0

*\*Subscripts (1, 2, 3); independent Fluctuate runs, each seeded with different random numbers, and 95% CIs were calculated by evaluating likelihood surfaces generated in each run. Values of  $\Theta_g$  and  $g$  (the exponential growth rate) are estimated simultaneously in a single Fluctuate run, and 95% CIs were generated from a normal distribution.*

**Table 4. Estimates of long-term inbreeding effective size,  $N_{ei}$ , for Rio Grande silvery minnow calculated with three mutation rates ( $\mu$ ) and values of  $\Theta$  (and 95% CIs) from Table 3.**

Mutation rate	Parameter Estimate	$N_{ei}$ <sup>a</sup>	-95% CI	+95%CI
$\mu^b = 6.5 \times 10^{-9}$	$\Theta_1$	$1.45 \times 10^6$	$1.16 \times 10^6$	$1.85 \times 10^6$
	$\Theta_2$	$1.41 \times 10^6$	$1.07 \times 10^6$	$1.94 \times 10^6$
	$\Theta_3$	$1.35 \times 10^6$	$1.01 \times 10^6$	$1.80 \times 10^6$
	$\Theta_g$	$2.50 \times 10^6$	$2.27 \times 10^6$	$2.73 \times 10^6$
$\mu^c = 1.0 \times 10^{-8}$	$\Theta_1$	$9.46 \times 10^5$	$7.56 \times 10^5$	$1.20 \times 10^6$
	$\Theta_2$	$9.14 \times 10^5$	$6.95 \times 10^5$	$1.26 \times 10^6$
	$\Theta_3$	$8.77 \times 10^5$	$6.59 \times 10^5$	$1.17 \times 10^6$
	$\Theta_g$	$1.62 \times 10^6$	$1.47 \times 10^6$	$1.77 \times 10^6$
$\mu^d = 2.7 \times 10^{-9}$	$\Theta_1$	$3.50 \times 10^6$	$2.80 \times 10^6$	$4.46 \times 10^6$
	$\Theta_2$	$3.39 \times 10^6$	$2.58 \times 10^6$	$4.66 \times 10^6$
	$\Theta_3$	$3.25 \times 10^6$	$2.44 \times 10^6$	$4.33 \times 10^6$
	$\Theta_g$	$6.01 \times 10^6$	$5.46 \times 10^6$	$6.56 \times 10^6$

<sup>a</sup>obtained by evaluating Eq. 1. and multiplying result by two.

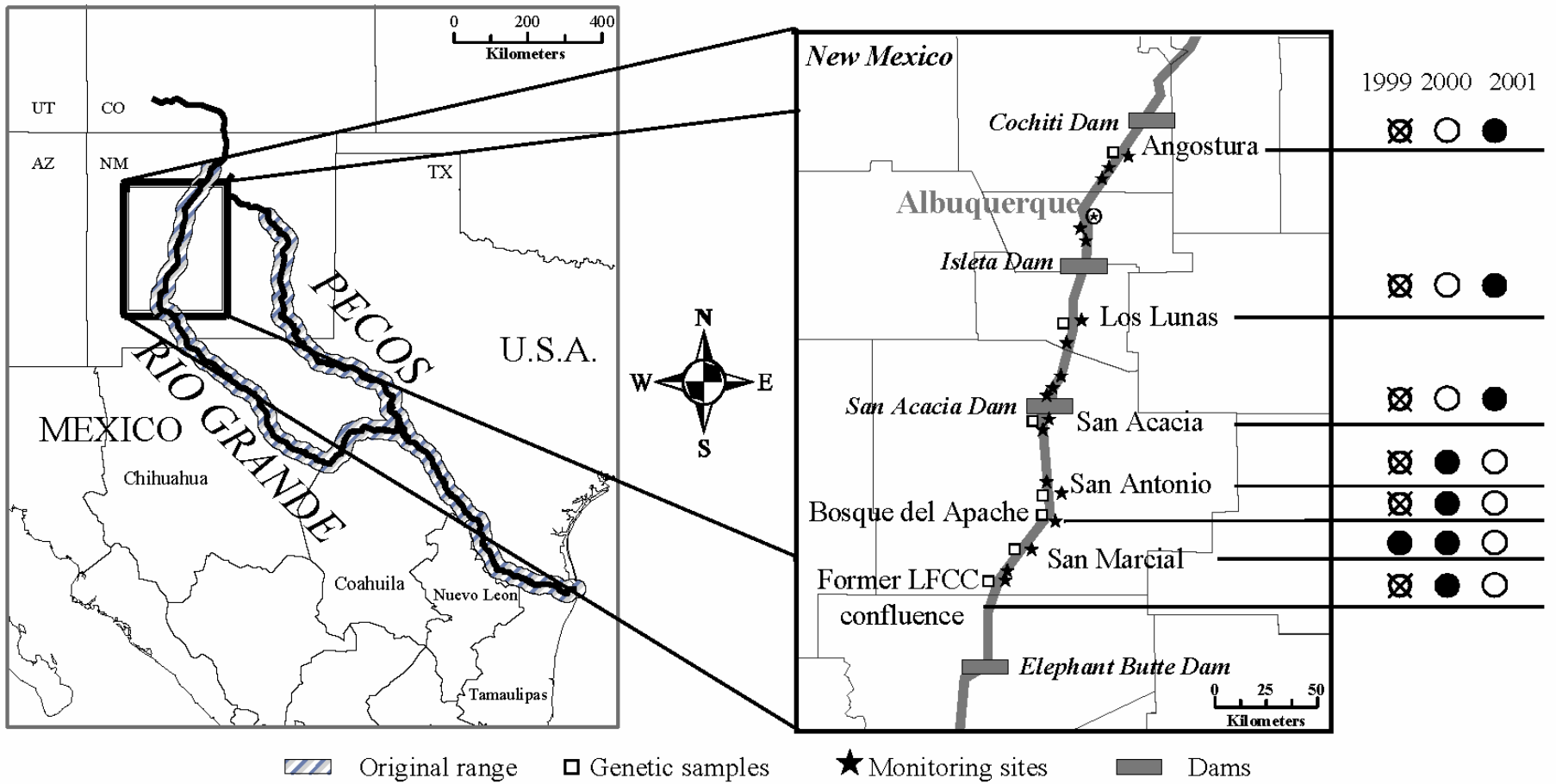
<sup>b</sup>protein-encoding mitochondrial genes, fishes (Bermingham et al. 1997).

<sup>c</sup>protein-encoding mitochondrial genes, mammals (Brown et al. 1979).

<sup>d</sup>lowest reported value for fishes (Sivasundar et al. 2001).

## Figure Legend

*Figure 1. Maps of historical and current geographic range (left) and population monitoring sites and genetic sampling localities in the middle Rio Grande, New Mexico (right). Sampling effort for genetic analysis (far right): solid circles, sampling events where > 20 individuals were taken at a locality; open circles, sampling events where insufficient numbers were taken for genetic analysis; circles with an X, no sampling.*



***Appendix. Population monitoring and estimation of adult census size (N)***

Estimates of adult numbers ( $N$ ) for this study were generated from Rio Grande silvery minnow population monitoring data (S. Platania & R. Dudley, unpublished data) obtained at 16 monitoring sites established in the middle Rio Grande from Angostura to Elephant Butte reservoir (Fig. 1). Detailed analysis of adult numbers focused on the San Acacia reach where eight monitoring sites are located (Fig. 1).

The geomorphology of the middle Rio Grande permits effective sampling by wading and seine netting. In the San Acacia reach, the river channel is relatively wide (average width = 181 m, SD = 131 m – P. Tashjian, personal communication) and shallow (almost always < 1.0 m). The braided river channel with sand and gravel substrate provides diverse aquatic habitats including main channel, shoreline runs, plunge pools with non-zero current velocity, and backwater habitats where current velocity is zero. Fishes were obtained using a 3 m × 1.5 m × 0.01 m (mesh) seine net, and the total distance seined in an individual seine haul was measured in meters. Approximately 17 seine hauls were conducted at each monitoring site, with individual seine hauls chosen to sample evenly across habitat types, but chosen haphazardly within habitat category.

Catch-per-unit-effort (CPUE) data for Rio Grande silvery minnow were used to estimate density (number of fish per meter squared) at each monitoring site by dividing the number of fish captured by the total area sampled (see Table below). Density estimates were checked visually for log normality by plotting log-transformed and untransformed data. Means and standard deviations were obtained by log-transforming density estimates at each site, using a normal distribution to estimate moments, and then back transforming.

Total adult numbers ( $N$ ) for the San Acacia reach were estimated as the product of mean density, length, and width of aquatic habitat in meters. Channel width is highly variable throughout the San Acacia reach and so  $N$  was estimated in three ways; (i) using mean channel width, (ii) using width = 50 m, which is equivalent to mean channel width minus 1 SD, and (iii) using zero channel width. Estimates of density, and thus estimates of  $N$ , have large confidence intervals because of high variability among monitoring sites. Standard deviation of density was roughly equivalent to the mean in all cases, and so lower bound 95% CI included zero. For this reason, we report  $\pm$  80% CIs for  $N$ .

**Appendix. Estimates of adult numbers (*N*) in the San Acacia reach of the middle Rio Grande based on population monitoring data obtained at eight sites from 1999 to 2001.**

Estimate <sup>a</sup>	1999	2000	2001	Summary Statistics
No. fishes sampled	701	366	54	1121 <sup>b</sup>
Area sampled (m <sup>2</sup> )	6256	8291	10124	24671 <sup>b</sup>
Mean Density (no./m <sup>2</sup> )	0.094	0.048	0.005	0.047 <sup>c</sup>
Density SD	0.087	0.037	0.005	0.060 <sup>c</sup>
<i>N</i> <sub>1</sub>	1.9 × 10 <sup>6</sup>	9.5 × 10 <sup>5</sup>	1.0 × 10 <sup>5</sup>	2.7 × 10 <sup>5d</sup>
-80% CI	4.1 × 10 <sup>4</sup>	2.0 × 10 <sup>5</sup>	1.2 × 10 <sup>4</sup>	
+80% CI	4.0 × 10 <sup>6</sup>	1.8 × 10 <sup>6</sup>	2.0 × 10 <sup>5</sup>	
<i>N</i> <sub>2</sub>	5.2 × 10 <sup>5</sup>	2.6 × 10 <sup>5</sup>	2.9 × 10 <sup>4</sup>	7.4 × 10 <sup>4d</sup>
-80% CI	1.1 × 10 <sup>4</sup>	5.6 × 10 <sup>4</sup>	3.4 × 10 <sup>3</sup>	
+80% CI	1.1 × 10 <sup>6</sup>	4.9 × 10 <sup>5</sup>	5.4 × 10 <sup>4</sup>	
<i>N</i> <sub>3</sub>	1.0 × 10 <sup>4</sup>	5.2 × 10 <sup>3</sup>	5.7 × 10 <sup>2</sup>	1.5 × 10 <sup>3d</sup>
-80% CI	2.2 × 10 <sup>2</sup>	1.1 × 10 <sup>3</sup>	6.8 × 10 <sup>1</sup>	
+80% CI	2.2 × 10 <sup>4</sup>	9.7 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	

<sup>a</sup>Estimates of *N*: *N*<sub>1</sub>, product of mean density, reach length (1.1 × 10<sup>5</sup> m), and average channel width (181 m); *N*<sub>2</sub>, product of mean density, reach length, and mean channel width minus 1 SD (= 50 m); *N*<sub>3</sub>, product of mean density and reach length.

<sup>b</sup> totals across years.

<sup>c</sup> computed across all samples assuming lognormal distribution.

<sup>d</sup> harmonic mean across years.