PROCEEDINGS OF THE ROYAL SOCIETY B
FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2006.3677
Published online

Life history and environmental variation interact to determine effective population to census size ratio

Thomas F. Turner*, Megan J. Osborne, Gregory R. Moyer†, Melissa A. Benavides and Dominique Alò

Department of Biology, and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131-0001, USA

Successful recovery and sustainability of threatened and exploited species depends in part on retention and maintenance of genetic diversity. Theory indicates that genetic diversity is lost at a rate inversely proportional to the genetically effective population size (N_e), which is roughly equal to one-half the adult census size (N) in many organisms. However, N_e has been reported to be up to five orders of magnitude lower than N in species with life histories that result in type III survivorship (high fecundity, but heavy mortality in early life stages, e.g. bony fishes), prompting speculation that low values of N_e may be a general feature of such organisms despite sometimes vast abundances. Here, we compared N_e and the ratio N_e/N across three ecologically similar fish species from the arid southwestern United States, all with type III life histories but with differing expectations of egg and larval survivorship that correlate with the degree of human-imposed habitat fragmentation. Our study indicates that type III life history may be necessary, but this alone is insufficient to account for extraordinarily low values of N_e/N. Rather, life history interacts with environmentally imposed mortality to determine the rate and magnitude of change in genetic diversity in these desert fish species.

Keywords: bottleneck; genetic diversity; fishing impacts; match-mismatch; type III survivorship; habitat fragmentation

1. INTRODUCTION

Many ecosystems have been adversely affected by human exploitation, which has resulted in alarming declines in abundance of some species. Mortality from direct removal of individuals (e.g. directed fishing), loss of suitable habitat, and fragmentation of remaining habitat into small and disconnected patches has direct demographic consequences for populations, namely, that migration, birth and death rates are altered. Sustained demographic change in the short term implies genetic change in the long term owing to the intimate connection of demographic and genetic processes (Avise 2000). A major challenge to conservation biology is to fully specify the linkage of demographic and genetic change so that prospects for species' survival and the outcomes of management and conservation plans can be accurately assessed.

Recent advances in theory (Caballero 1994; Wang & Caballero 1999) and analytical tools (Pearse & Crandall 2004) have allowed increasingly sophisticated description of relationships between demographic and genetic processes in different kinds of organisms. One approach is to evaluate the ratio of genetically effective population size and census size owing to the explicit connection of demographic and genetic processes represented in the ratio (Nunney & Elam 1994; Frankham 1995). Effective size (N_e) is arguably the most important population parameter in evolutionary biology because it determines, among other things, the rate at which the genetic diversity is expected to be lost at each generation. The adult census size (N) is a parameter of fundamental interest in demographic studies. A key theoretical result is that $N_e/N = 0.5$ over a broad range of mating systems and life-history characteristics known to influence this ratio (Nunney & Elam 1994) in an otherwise demographically stable and closed population. Mean empirical estimates of N_e/N (based largely on observations from birds and mammals) are around 0.1, but reach 0.5 after correction for fluctuation of N (Frankham 1995; Vucetich *et al.* 1997; but see Waples 2002*a*). Taken together, these results imply that N_e can be viewed as a relatively simple function of N and, more importantly, that expected rates of genetic change in conserved and managed populations can be predicted by having an accurate estimate of N.

There are a number of species where estimated N_e/N is several orders of magnitude lower than expectation and the discrepancy cannot be accounted for by fluctuations of N (Hedgecock 1994; Hauser *et al.* 2002; Turner *et al.* 2002; Hutchinson *et al.* 2003, but see Poulsen *et al.* 2006). The apparent disconnection between demographic and genetic processes usually involve species characterized by enormous fecundity but low parental investment per

* Author for correspondence (turnert@unm.edu).
† Present address: Hatfield Marine Science Centre, Oregon State University, Coastal Oregon Marine Experiment Station, Newport, OR 97365-5296, USA

The electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2006.3677 or via http://www.journals.royalsoc.ac.uk.

Received 20 June 2006
Accepted 15 July 2006

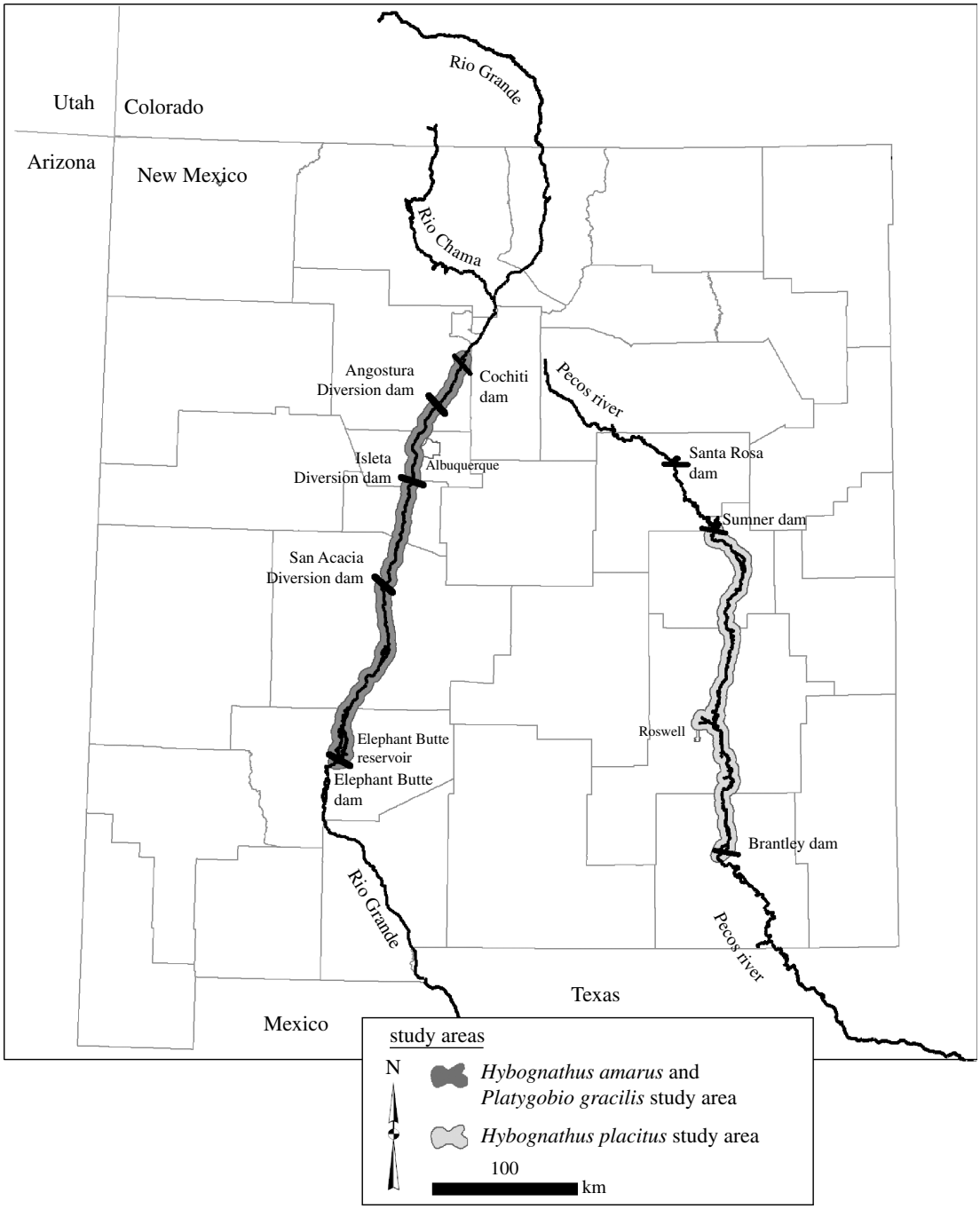

Figure 1. Geographic sampling areas of three cyprinid fish species and water diversion dams (indicated by dark horizontal lines) in the Rio Grande and Pecos River, New Mexico.

offspring, and consequently high mortality in early life stages (termed type III survivorship). It has been proposed that such life histories (termed herein as 'type III life histories') could result in extremely high variance in reproductive success among individuals, especially when propagules are passively distributed into a heterogeneous environment (Hedgecock 1994). In this scenario, termed 'sweepstakes' or 'match–mismatch' recruitment (Hedgecock 1994; Flowers *et al.* 2002), very few breeding pairs contribute the majority of offspring to the next generation, resulting in lowered N_e but with little effect on N because each successful breeder can potentially contribute large numbers of offspring.

Are values of $N_e/N \ll 0.5$ generally expected in organisms with type III survivorship? Alternatively, are N_e/N values close to those predicted by theory when sweepstakes recruitment is not obviously acting in an otherwise type III species? To address these questions, we compared N_e and N_e/N across three ecologically similar fish species in a river system in the southwestern United States, all with life histories that could result in type III survivorship, but with differing expectations of egg and larval survivorship that correlate with the degree of human-imposed habitat fragmentation. Our results indicate that type III survivorship may be necessary, but this alone is insufficient to account for extraordinarily low values of N_e/N. Rather, type III life history and environmentally imposed early mortality interact to determine the rate and magnitude of change in genetic diversity.

Table 1. Life history and ecological attributes of the study species in the middle Rio Grande from unpublished and published sources (Sublette *et al.* 1990; Taylor & Miller 1990).

species	maximum body length (mm)	generation time, *G* (years)	life span (years)	age at maturity (years)	distribution	river course	egg/larval dispersal
Hybognathus amarus	90	1.22	2	1	Rio Grande	fragmented	pelagic
Hybognathus placitus	95	1.35	3	1	Pecos River	unfragmented	pelagic
Platygobio gracilis	150	2.37	4	2	Rio Grande	fragmented	sessile

2. MATERIAL AND METHODS

(a) *Study site*

This study was conducted in the Rio Grande and its major tributary, the Pecos River (figure 1). Together, these rivers support the overwhelming majority of the human population in New Mexico and, not surprisingly, their flows are regulated by dams designed to control flood and to store and extract water for cities and agriculture. Climatic conditions are arid and annual precipitation (less than 25 cm) does not differ significantly between basins (*t*-test performed on data available at http://waterdata.usgs.gov). River discharge is largely determined by spring snowmelt and summer monsoon rains. Extensive drying and intermittent flows are common in both basins, especially during drought cycles driven in part by El Niño and Pacific Decadal climatic oscillations (Sheppard *et al.* 2002). A 280 km river reach of the Rio Grande was sampled for fishes (details of sampling localities are provided in Moyer *et al.* (2005) and Osborne *et al.* (2005)). This reach, known as the middle Rio Grande, is fragmented by five dams (figure 1) that are impassable to fishes moving upstream, but not downstream. An unfragmented, but otherwise similar 332 km reach was sampled in the Pecos River (figure 1).

Ichthyofaunal composition of the Rio Grande and Pecos River, like other major rivers in the southwestern United States, is dominated by members of the freshwater family Cyprinidae (Sublette *et al.* 1990). Our study focused on three ecologically similar cyprinid fish species: the federally endangered and endemic Rio Grande silvery minnow, *Hybognathus amarus*; its congener, the plains minnow, *Hybognathus placitus*; and a closely related and co-occurring minnow, the flathead chub, *Platygobio gracilis*. All three species are short-lived, small-bodied, have very high fecundity, produce small eggs (approx. 1 mm diameter) and poorly developed offspring at hatching (table 1). There is no obvious sexual dimorphism in these species and sex ratios do not deviate from 1 : 1 (S. P. Platania 2005, personal communication; Taylor & Miller 1990; T. F. Turner 2005, unpublished data).

Despite overall ecological similarity, these species differ in key early life-history traits. *Hybognathus* sp. are characterized by pelagic early life history, i.e. eggs and larvae are semi-buoyant and drift passively downstream with river currents (Platania & Altenbach 1998). *Platygobio gracilis* produces 'sticky' eggs that sink and attach to the substrate, and larvae do not appear to drift long distances in the Rio Grande. Species were chosen to provide two contrasts: (i) among species with pelagic early life history in fragmented versus unfragmented river environments (*H. amarus* versus *H. placitus*); and (ii) among species co-inhabiting a fragmented environment but with pelagic versus sessile early life history (*H. amarus* versus *P. gracilis*).

(b) *Molecular work*

We collected whole fishes or fin clip samples for genetic analysis from 1999 to 2003 (table 2). Representative samples were screened for genetic variation at eight microsatellite loci: *CA6* (Dimsoski *et al.* 2000), *Lco3*, *Lco4*, *Lco5*, *Lco6*, *Lco7* (Turner *et al.* 2004), *Ppro118 and Ppro126* (Bessert & Ortí 2003). Based on initial screening, four loci were chosen for each species that were consistently scorable and reproducible over multiple assays. Individual variation was also characterized at an approximately 295 base pair fragment of the protein-encoding mitochondrial (mt)DNA–*ND4* locus. Microsatellite and mtDNA loci were amplified via polymerase chain reaction following previously published protocols (Bessert & Ortí 2003; Alò & Turner 2005; Moyer *et al.* 2005). Microsatellite genotypes were characterized with an automated sequencer (ABI 377, Applied Bioscience) equipped with GENESCAN software (Applied Bioscience). Haplotypic variation in the mtDNA–*ND4* fragment was determined by single-stranded conformational polymorphism (SSCP) analysis (Sunnucks *et al.* 2000). Haplotype scores from SSCP were verified by direct nucleotide sequencing of a representative subset of individual DNA samples (approx. 25%).

(c) *Statistical analysis*

Microsatellite allele and genotype frequencies, expected heterozygosities ($H_e \pm$s.d.), and other summary statistics were tabulated with the MICROSATELLITE TOOLKIT v. 3.1 (add-in for MICROSOFT EXCEL, written by S. Park, available at http://oscar.gen.tcd.ie/~sdepark/ms-toolkit/). For mtDNA, gene diversities ($h \pm$s.d.; Nei 1987) and haplotypic richness were calculated with FSTAT v. 2.9.3 (Goudet 1995). Each sample was tested for departure from Hardy–Weinberg (HW) equilibrium expectations by comparing observed inbreeding coefficients (F_{IS}) to a distribution of 1000 bootstrap replicates, and linkage disequilibrium was tested across all pairs of loci with FSTAT. In cases where significant deviation from HW equilibrium was detected, we used the program MICROCHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004) to evaluate the probable cause of deviation (e.g. null alleles, miss-scoring owing to stuttering, etc.). Population substructure was examined with microsatellite data by computing F_{ST} (Weir & Cockerham 1984) among sampling localities where $n > 20$.

Variance genetic effective size (N_e) and 95% confidence intervals (CIs) were estimated from temporal changes in microsatellite allele frequencies across year classes using the so-called temporal method (Nei & Tajima 1981; Waples 1989; Jorde & Ryman 1995) and a pseudo-maximum-likelihood procedure implemented in the program MLNE v. 2.3 (Wang 2001). For mtDNA data (analysed separately), variance effective size for the female portion of the population

Table 2. Measures of genetic variability at microsatellite and mtDNA loci. (n, number of individuals sampled; F_{IS}, Inbreeding coefficient; H_e, unbiased Hardy–Weinberg expected heterozygosity; N_a, allelic richness adjusted to the smallest sample size in the comparison; h, Nei's gene diversity; N_i, estimate of yearly adult census numbers. Raw microsatellite and mtDNA data are provided in the electronic supplementary material. $^*F_{IS}$ is significantly different from zero.)

		Hybognathus amarus					*Hybognathus placitus*			*Platygobio gracilis*	
locus		1999	2000	2001	2002	2003	1999	2002	2003	2001	2002
CA6	n	33	187	121	387	168	99	140	157	—	—
	F_{IS}	0.31	−0.05	0.06	0.10*	−0.09	0.05	−0.09	−0.03	—	—
	H_e	0.75	0.65	0.71	0.79	0.78	0.87	0.88	0.88	—	—
	N_a	8.00	6.94	7.45	10.23	8.32	7.00	9.82	10.06	—	—
Lco3	n	44	194	126	374	169	99	139	161	—	—
	F_{IS}	−0.01	−0.02	0.11	−0.02	−0.07	0.02	−0.03	−0.01	—	—
	H_e	0.79	0.75	0.70	0.78	0.79	0.80	0.81	0.79	—	—
	N_a	7.50	7.61	8.16	9.43	8.09	7.00	8.30	8.07	—	—
Lco4	n	—	—	—	—	—	—	—	—	73	144
	F_{IS}	—	—	—	—	—	—	—	—	0.39*	0.37*
	H_e	—	—	—	—	—	—	—	—	0.52	0.46
	N_a	—	—	—	—	—	—	—	—	4.74	4.16
Lco5	n	—	—	—	—	—	—	—	—	72	142
	F_{IS}	—	—	—	—	—	—	—	—	−0.02	0.00
	H_e	—	—	—	—	—	—	—	—	0.82	0.81
	N_a	—	—	—	—	—	—	—	—	11.63	10.64
Lco6	n	41	193	127	362	165	99	141	156	—	—
	F_{IS}	0.14	0.06	0.04	0.26*	0.10	0.02	0.21*	0.15*	—	—
	H_e	0.71	0.67	0.70	0.62	0.54	0.72	0.71	0.72	—	—
	N_a	9.38	10.06	9.84	9.69	8.54	7.00	7.31	7.34	—	—
Lco7	n	39	192	126	382	166	98	141	160	—	—
	F_{IS}	0.11	0.10*	0.13*	0.35*	0.10	0.01	0.14*	0.13*	—	—
	H_e	0.78	0.84	0.80	0.81	0.79	0.87	0.88	0.88	—	—
	N_a	6.69	10.39	9.11	10.08	9.78	13.97	14.18	15.69	—	—
Ppro118	n	—	—	—	—	—	—	—	—	71	138
	F_{IS}	—	—	—	—	—	—	—	—	0.051	0.036
	H_e	—	—	—	—	—	—	—	—	0.89	0.92
	N_a	—	—	—	—	—	—	—	—	19.2	21.0
Ppro126	n	—	—	—	—	—	—	—	—	64	135
	F_{IS}	—	—	—	—	—	—	—	—	−0.15	−0.13
	H_e	—	—	—	—	—	—	—	—	0.68	0.77
	N_a	—	—	—	—	—	—	—	—	8.00	8.00
ND4	n	34	130	99	377	168	66	145	148	73	141
	h	0.69	0.41	0.65	0.64	0.53	0.80	0.71	0.75	0.74	0.74
	N_a	6.00	5.57	7.10	5.76	6.95	23.00	21.71	22.94	5.75	5.84
N_i		7.3×10^5	3.4×10^5	3.7×10^4	1.8×10^5	3.0×10^4	1.7×10^5	3.3×10^5	3.5×10^5	3.0×10^4	1.2×10^4

(N_{ef}) was estimated with the temporal method and MLNE. Sampling localities were pooled by year class prior to analysis. We assumed that genetic sampling did not change the available pool of reproductive individuals and that migration from outside the study area did not affect estimates of N_e. Upstream migration is negligible because fish movement is precluded by dams and these species are rarely taken upstream of the study area.

Estimates of N_e from the temporal method and MLNE were corrected for effects of overlapping generations using equations in Jorde & Ryman (1995) and life table data obtained separately (see electronic supplementary material) to estimate a correction factor C and the generation time G. The model accounts for effects of genetic drift as a cohort passes from one age class to the next and for genetic contributions of parents from multiple age classes to progeny in a stationary (non-growing) population. For temporal-method estimation, we substituted the quantities C, G and $\bar{F}'$(average standardized variance of allele frequencies, corrected for sampling variance) into eqn 4 in Jorde & Ryman (1996) and solved to yield N_e. This equation was modified for estimation of N_{ef} following Turner *et al.* (1999). Estimates of N_e from MLNE were multiplied by the ratio C/G to correct for overlapping generations.

Temporal-method estimates of N_e and N_{ef} were calculated from F' values obtained from temporally adjacent pairs of cohorts for all species (Jorde & Ryman 1996). MLNE estimates were based on temporally adjacent cohorts except we included the 1999 *H. placitus* sample in estimation. *Hybognathus* sp. have essentially non-overlapping generations; consequently, corrected and uncorrected estimates of N_e do not differ appreciably under any method of estimation.

For each year that genetic data were collected, adult census size in the ith year (N_i) was estimated from data obtained from population monitoring studies in the Rio Grande and Pecos River (data available from the Division of Fishes, Museum of Southwestern Biology). Raw data were

the number of fish captured per area sampled at up to 20 population-monitoring sites in each river, taken in the two months preceding reproduction. The total number of individuals (N_{itotal}) was estimated as the product of mean density (calculated across all samples), river reach length and mean -1 s.d. river channel width to account for unusually low flows during the study (for additional discussion see appendix in Alò & Turner (2005). For *Hybognathus* sp., $N_{itotal} \approx N_i$ because nearly all individuals sampled were reproductively capable adults. *Platygobio gracilis* lives longer and matures later than *Hybognathus*, so N_{itotal} was multiplied by 0.17, the expected fraction of reproductive adults in the sample, to obtain N_i (see electronic supplementary material). The expected fraction of reproductive adults was determined by first evaluating size (and age) at first reproduction for *Platygobio* in the Rio Grande, and then determining the mean fraction of individuals that equalled or exceeded this size in three datasets: two from the study area in the Rio Grande (sample year 1993, $n=253$, fraction of reproductive adults $=0.23$; sample years 1999, 2002 and 2003, $n=316$, fraction of reproductive adults $=0.15$), and one from upper Missouri and Yellowstone Rivers in North Dakota ($n=1254$, fraction of reproductive adults $=0.14$; Welker & Scarnecchia 2004).

The ratio N_e/N was computed by dividing the estimate of N_e by arithmetic and harmonic mean N_i for each species. Annual adult census sizes for *P. gracilis* and *H. placitus* were relatively stable across sample years. For *H. amarus*, N_i declined by an order of magnitude, but was not less than 10^4 individuals over the study period.

3. RESULTS

Gene diversities at microsatellite loci (measured as H_e) were nearly constant over the study period for *P. gracilis* and *H. placitus*. Similarly, diversity at the mtDNA–ND4 locus (measured as h) was constant for *P. gracilis* and fluctuated slightly for *H. placitus* (figure 2). In contrast, H_e declined in *H. amarus* over the study period ($H_e=0.81$ in 1999, $H_e=0.75$ in 2003; figure 2). Gene diversity at the mtDNA–*ND4* locus declined substantially ($h=0.69$ in 1999, $h=0.53$ in 2003) over the study period in this species (figure 2).

Deviations from HW equilibrium were detected in 11 (5, *H. amarus*; 4, *H. placitus*; and 2, *P. gracilis*) out of 40 tests after Bonferroni correction with nominal $\alpha=0.05$. All deviations resulted from heterozygote deficiencies as indicated by significantly positive F_{IS} values (table 2). *Post hoc* analysis with MICROCHECKER indicated that null alleles were the probable cause of deviation from HW equilibrium in all cases. There was no evidence of linkage disequilibrium among microsatellite loci for any species.

Significant spatial genetic structure was not detected in any of the following species: *H. amarus* ($F_{ST}=-0.0025$ ns; based on four geographically distinct localities sampled in 2000), *H. placitus* ($F_{ST}=-0.001$ ns; four localities, 2002) and *P. gracilis* ($F_{ST}=0.000$ ns; four localities, 2002). Values of F_{ST} near zero are consistent with high gene flow among sampling localities for all species.

The magnitude of temporal shifts of microsatellite allele frequencies differed among species as reflected in values of N_e. After correction for overlapping generations, point estimates of variance effective size from the temporal method and MLNE were consistent and indicated that *H. amarus* $N_e \ll$ *P. gracilis* $N_e \ll$ *H. placitus* N_e (table 3).

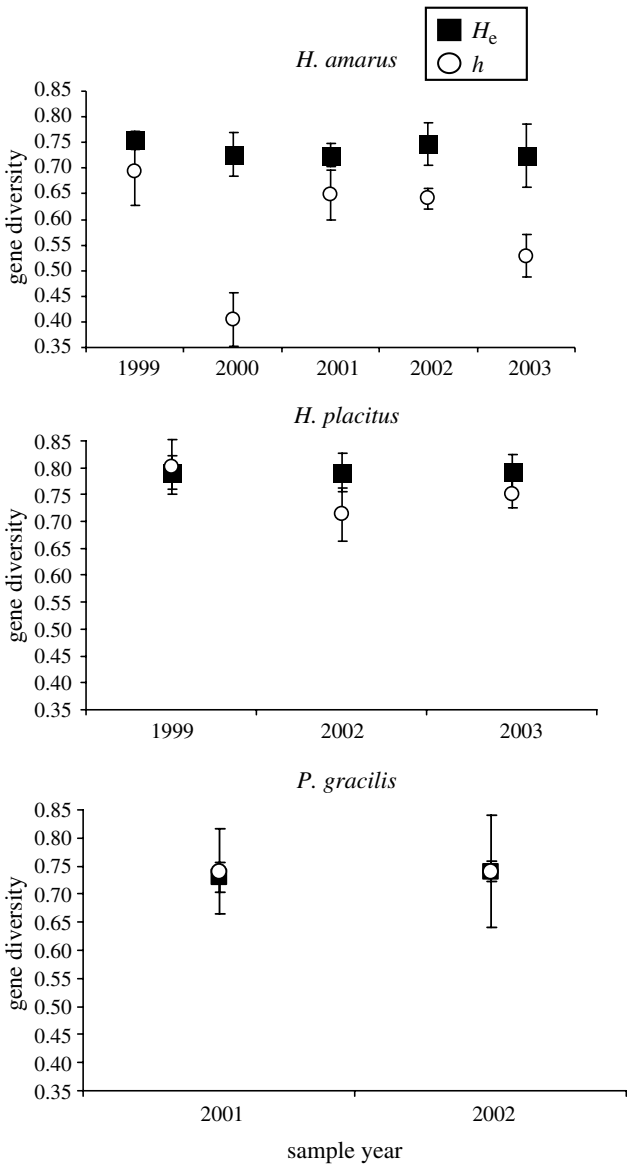

Figure 2. Gene diversities for microsatellites (H_e) and mtDNA haplotypes (h) (bars represent $\pm$s.d.) for each study species plotted by year class.

For *H. placitus*, the estimate of N_e can be considered to be very large that it is indistinguishable from an effectively infinite population, given the molecular markers and sample sizes obtained (Waples 1989). The estimated value of N_{ef} based on temporal-method and MLNE analyses of mtDNA–*ND4* haplotype frequencies revealed a pattern similar to microsatellites, namely *H. amarus* N_{ef} < *P. gracilis* $N_{ef} \ll$ *H. placitus* N_{ef} (table 3). In general, N_{ef} was estimated with lower precision than N_e (as revealed by broader 95% CIs; figure 3) because the estimate is based on a single locus with fewer independent alleles than microsatellites (Waples 1989).

We evaluated potential effects on our estimates of N_e by adjusting allele frequencies under the assumption that one or more null alleles were present (algorithm of Van Oosterhaut *et al.* 2004), and then reanalysing adjusted frequency data. We obtained the following values from temporal-method estimation: *H. amarus* frequency-adjusted $N_e=75$ (95% CIs: 34, 204), *P. gracilis* frequency-adjusted $N_e=989$ (184, ∞) and *H. placitus* frequency-adjusted $N_e=\infty$ (257, ∞). For

Table 3. Arithmetic mean adult census size ($\bar{N}_i$), harmonic mean adult census size ($\tilde{N}_i$), correction factors for overlapping generations (C), mean generation time (G), temporal-method estimates of N_e and N_{ef} (following Jorde & Ryman 1996, indicated as J & R), pseudo-maximum-likelihood estimates of N_e and N_{ef} (following Wang 2001, indicated as MLNE) and ratios of N_e/N for each study species. (Values of C and G were determined by using methods in Jorde & Ryman (1995, 1996) and static life tables for each species from the electronic supplementary material.)

	species		
estimate	*Hybognathus amarus*	*Hybognathus placitus*	*Platygobio gracilis*
$\bar{N}_i$	2.6×10^5	2.8×10^5	2.1×10^4
$\tilde{N}_i$	7.1×10^4	2.5×10^5	1.7×10^4
C	1.55	2.10	7.10
G	1.22	1.35	2.37
J & R-N_e	90	>50 000	812
(±95% CIs)	(34, 186)	(177, ∞)	(171, ∞)
J & R-N_{ef}	28	>50 000	190
(±95% CIs)	(5, 108)	(3827, ∞)	(23, ∞)
MLNE-N_e	277	>50 000	5395
(±95% CIs)	(226, 353)	(3522, ∞)	(481, ∞)
MLNE-N_{ef}	202	>50 000	356
(±95% CIs	(111, 544)	(330, ∞)	(69, ∞)
J & R-$N_e/\bar{N}_i$	0.0003	>0.179	0.039
J & R-$N_e/\tilde{N}_i$	0.001	>0.200	0.048
MLNE-$N_e/\bar{N}_i$	0.001	>0.179	0.260
MLNE-$N_e/\tilde{N}_i$	0.004	>0.200	0.317

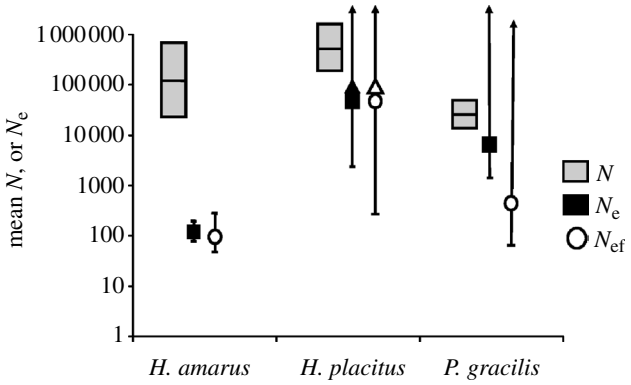

Figure 3. Range (shaded box) of adult census size for each year class (N_i), arithmetic mean $N_i=N$ (midline of shaded box), variance effective size (N_e) ±95% CIs, and variance female effective size (N_{ef}) ±95% CIs estimated from MLNE for three cyprinid fish species. Upward arrow indicates estimates of N_e or N_{ef} that exceed 50 000 and/or an upper 95% CIs that are infinitely large.

MLNE, we obtained *H. amarus* frequency-adjusted $N_e=249$ (193, 334), *P. gracilis* frequency-adjusted $N_e=5119$ (164, ∞) and *H. placitus* frequency-adjusted $N_e>50\,000$ (2257, ∞).

Arithmetic mean adult numbers (N) were estimated to be roughly 10^4 for *P. gracilis* and an order of magnitude larger for *Hybognathus* sp. (table 3; figure 3). Estimated N_e/N was greater than or equal to approximately 0.04 for *H. placitus* and *P. gracilis*, but estimated N_e/N was less than 0.005 for *H. amarus* (table 3; figure 3) under all conditions we evaluated.

4. DISCUSSION

There are a number of ways to estimate and interpret N_e, but in this study it represents the present-day variance effective population size, i.e. the number of ideal breeding individuals in each generation that would produce the observed temporal shift in allele frequencies over the study period. The term 'ideal' refers to conditions of the Wright–Fisher idealized population, which include 1 : 1 sex ratio, panmixia, discrete generations, Poisson-distributed variance in reproductive success among breeding individuals and stable N (Caballero 1994; Wang & Caballero 1999). We interpreted the ratio N_e/N to represent the cumulative reduction of N_e over the study period attributable to variance in reproductive success in excess of Poisson variance. This is because our study species either met other ideal conditions (e.g. equal sex ratio, no appreciable population structure) or we explicitly incorporated effects of violations of other ideal conditions (e.g. correction of N_e for overlapping generations in all species; see electronic supplementary material) into the estimate of N_e/N (e.g. Rowe & Beebee 2004).

Of three species examined, only *H. amarus* exhibits N_e/N that is substantially lower than expectation. This species exhibits type III survivorship, pelagic early life history, and occurs in a highly fragmented river reach. Genetic and ecological data obtained for drifting eggs (Dudley 2004; Osborne *et al.* 2005) and breeding adults (Alò & Turner 2005) are consistent with the idea that reproductive output from most breeding pairs is lost from mortality or emigration as eggs and larvae are transported downstream through dams, resulting in high variance in reproductive success and low N_e/N. Even if larvae survive entrainment, mortality from desiccation occurs because the reach downstream of San Acacia dam (figure 1) is subject to substantial drying most summers. Drifting eggs maintain genetic 'cohesion' as they drift downstream (Osborne *et al.* 2005), which results in differential (i.e. family correlated) mortality and enhances variance in reproductive success (Waples 2002*b*). The probability of egg retention in the natal river reach is probably related to the distance to the nearest downstream dam and the magnitude of river flows where spawning occurred (Dudley 2004).

Extraordinarily low N_e/N was not observed for *H. placitus*, a species with nearly identical life history and ecology to *H. amarus*. This species occupies an unfragmented portion of the Pecos River. Our results suggest that differential loss of reproductive output may not occur in the Pecos River to the extent it occurs on the Rio Grande. Consequently, it appears that variance in reproductive success is greatly reduced despite type III survivorship and pelagic early life history.

River fragmentation and type III life history cannot completely account for low values of N_e/N. *Platygobio gracilis* co-occurs with *H. amarus*, has similar N, but exhibits 10-fold greater N_e. This contrast suggests that sessile early life history diminishes the effect of river fragmentation on variance in reproductive success in *P. gracilis* by limiting downstream transport of reproductive output. Taken together, these contrasts indicate that an interaction of type III survivorship, pelagic life history, and a mechanism that results in heavy but differential mortality (in this case habitat fragmentation) is required to generate very low values of N_e/N. This is not to say that fragmentation does not affect *Platygobio*, but that the expected rate of decline in genetic diversity is lower in *P. gracilis* than in *H. amarus*. The estimate of N_{ef} from MLNE was eightfold lower than expected (based on an expectation of $0.5N_e$) for *P. gracilis*, and may reflect higher variance of reproductive success in females compared to males over the study period. However, mtDNA sampled over a longer (but non-sequential) time-series indicated that $N_{ef} > 50\,000$ for this species.

Low variance N_e translates to a higher rate of loss of genetic diversity in *H. amarus* than in other species over the study period. In the case of mtDNA, genetic diversity (h) in 2003 was substantially lower than in 1999. However, it is possible that management practices implemented over the study influenced our results. Beginning in 2002, a large-scale captive rearing and population supplementation program was initiated by the US Fish and Wildlife Service. In this program, fertilized eggs are recovered from the Rio Grande as they drift downstream, reared to adulthood in hatcheries, and then repatriated as adults prior to spawning the next year. The estimate of N_e measured prior to hatchery supplementation (between 1999 and 2001) was roughly 80 (based on seven microsatellite loci; Alò & Turner 2005) and 95% CIs overlap the present estimate, suggesting that population supplementation did not strongly affect estimates of N_e in this study. In contrast to *H. amarus*, genetic diversity measures for *H. placitus* or *P. gracilis* were relatively unchanged over the study period.

The ratio N_e/N, as estimated and defined in this study, has potentially important applications in formulation of species management and conservation plans, and it is beginning to be used in risk assessment of commercially exploited species (Dulvy *et al.* 2004; Hutchings & Reynolds 2004). The approach has some distinct advantages. For example, variance N_e estimated from temporal shifts of allele frequencies is insensitive to historical population conditions (i.e. past population bottlenecks) and largely reflects current population dynamics of the focal species (Husband & Barrett 1992). Human-mediated disturbance usually happens in ecological, not evolutionary, time—although genetic consequences persist in evolutionary time—and so the temporal method permits evaluation of species' response at relevant time-scales. The ratio is especially useful for comparative studies (e.g. fished versus unfished populations; Hauser *et al.* 2002) because effects attributable to species-specific idiosyncrasies of population history are negligible.

Some concerns remain regarding the estimation of N_e/N. First, there are potential technical errors due to mis-scoring and often the presence of null alleles in molecular datasets, especially microsatellites. Significant deviations from HW equilibrium were evident for all three species in this study, and null alleles were probably present in all loci that deviated significantly from HW equilibrium. Analysis of adjusted allele frequencies (in MICROCHECKER) yielded similar estimates of N_e, and thus we concluded that comparative results are robust to the presence of null alleles. A second concern is that there is potential for bias in sampling of study individuals. If samples are not random, then temporal-method analysis is expected to produce biased estimates of N_e and N_e/N. In our study, sampling was done in nearly identical fashion across species. Adult (but not early) life histories and ecologies are also very similar among species. If bias owing to non-random sampling is present, then it may act in similar fashion across species in the comparison. However, if we had sampled at an earlier life stage (e.g. eggs or larvae) for genetic analysis, bias may have differentially affected our estimates which would make comparisons among species more problematic. Any comparative study of this kind is subject to unknown ecological differences among study taxa which could affect sampling and bias results (Waples 2002*a*).

There are also some theoretical complications in relating N_e to N in species with overlapping generations. For example, Jorde & Ryman's (1995) approach assumes constant population size each year, but in our study there is some fluctuation in yearly estimates of census size. In addition, estimation methods for N_e differ between the temporal method and MLNE, where the latter method was designed for discrete generations (Wang 2001). *Hybognathus* sp. are characterized by nearly discrete generations (i.e. $C/G \approx 1$), but *P. gracilis* has overlapping generations. We opted to estimate N_e for all species with both analytical methods, and used arithmetic and harmonic mean adult census sizes in estimation of N_e/N (cf. Kalinowski & Waples 2002; Waples 2005). The general pattern that emerges among species is the same regardless of the estimation approach, but actual estimates of N_e/N differ between methods, especially for *P. gracilis*. Thus, point estimates of N_e/N should be viewed as rough approximations, especially in light of substantial uncertainty in estimation of N_e and N.

It would be very useful to develop a general rule of thumb regarding the values that N_e/N is expected to take in species with type III survivorship, especially for species like commercially exploited marine fishes where estimates of N from commercial catches are often more readily available than estimates of N_e. Our results suggest that N_e/N is expected to be very small only when some extrinsic mechanism (e.g. overfishing (Hauser *et al.* 2002; Hutchinson *et al.* 2003), variance in productivity among habitats (Turner *et al.* 2002), and/or habitat-induced early mortality (this study)) enhances variance in reproductive success among individuals. When such conditions are not present, values of N_e/N appear to be similar to empirically

derived values obtained for mammals, birds and other organisms that lack life histories that promote type III survivorship.

Funding was provided by Middle Rio Grande Endangered Species Collaborative Program, the US Fish and Wildlife Service and the National Science Foundation (NSF; DEB-0133233). M.A.B. was supported by the NSF Undergraduate Mentoring in Environmental Biology Program (DEB-0102773). Opinions, findings and conclusions or recommendations expressed are those of the authors and do not necessarily reflect the views of the NSF. We thank the Museum of Southwestern Biology (MSB) staff and students, especially S. P. Platania, R. K. Dudley and A. M. Snyder, for technical advice, assistance and support. Figure 1 was prepared by S. J. Gottlieb. R. S. Waples and an anonymous reviewer provided comments and suggestions that considerably improved the manuscript. Collecting activities were conducted under US permit numbers TE001623-0 and TE038055-0, and New Mexico State permit numbers 1896 and 3015. Materials examined in this research were deposited in the Division of Fishes, MSB. Nucleotide sequences were deposited in GenBank under accession numbers AY854251–AY854260.

REFERENCES

Alò, D. & Turner, T. F. 2005 Effects of habitat fragmentation on effective population size in the endangered Rio Grande silvery minnow. *Conserv. Biol.* **19**, 1138–1148. (doi:10.1111/j.1523-1739.2005.00081.x)

Avise, J. C. 2000 *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Bessert, M. L. & Ortí, G. 2003 Microsatellite loci for paternity analysis in the fathead minnow, *Pimephales promelas* (Teleostei: Cyprinidae). *Mol. Ecol. Notes* **3**, 532–534. (doi:10.1046/j.1471-8286.2003.00501.x)

Caballero, A. 1994 Developments in the prediction of effective population size. *Heredity* **73**, 657–679.

Dimsoski, P., Toth, G. & Bagley, M. 2000 Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). *Mol. Ecol.* **9**, 2187–2189.

Dudley, R. K. 2004 Ichthyofaunal drift in fragmented rivers: empirically-based models and conservation implications. Ph.D. thesis, University of New Mexico, Albuquerque, NM.

Dulvy, N. K., Ellis, J. R., Goodwin, N. B., Grant, A., Reynolds, J. D. & Jennings, S. 2004 Methods of assessing extinction risk in marine fishes. *Fish Fish.* **5**, 255–276.

Flowers, J. M., Schroeter, S. C. & Burton, R. S. 2002 The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution* **56**, 1445–1453. (doi:10.1554/0014-3820(2002)056[1445:TRSHMW]2.0.CO;2)

Frankham, R. 1995 Effective population size/adult population size ratios in wildlife—a review. *Genet. Res.* **66**, 95–107.

Goudet, J. 1995 FSTAT (Version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**, 485–486.

Hauser, L., Adcock, G. J., Smith, P. J., Ramirez, J. H. B. & Carvalho, G. R. 2002 Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proc. Natl Acad. Sci. USA* **99**, 11 742–11 747. (doi:10.1073/pnas.172242899)

Hedgecock, D. 1994 Does variance in reproductive success limit effective population sizes of marine organisms? In *Genetics and evolution of aquatic organisms* (ed. A. R. Beaumont), pp. 122–134. London, UK: Chapman & Hall.

Husband, B. C. & Barrett, S. C. H. 1992 Effective population size and genetic drift in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* **46**, 1875–1890. (doi:10.2307/2410037)

Hutchings, J. A. & Reynolds, J. D. 2004 Marine fish population collapses: consequences for recovery and extinction risk. *Bioscience* **54**, 297–309. (doi:10.1641/0006-3568(2004)054[0297:MFPCCF]2.0.CO;2)

Hutchinson, W. F., van Oosterhout, C., Rogers, S. I. & Carvalho, G. R. 2003 Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proc. R. Soc. B* **270**, 2125–2132. (doi:10.1098/rspb.2003.2493)

Jorde, P. E. & Ryman, N. 1995 Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics* **139**, 1077–1090.

Jorde, P. E. & Ryman, N. 1996 Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics* **143**, 1369–1381.

Kalinowski, S. T. & Waples, R. S. 2002 Relationship of effective to census size in fluctuating populations. *Conserv. Biol.* **16**, 129–136. (doi:10.1046/j.1523-1739.2002.00134.x)

Moyer, G. R., Osborne, M. J. & Turner, T. F. 2005 Genetic and ecological dynamics of species replacement in an arid-land river. *Mol. Ecol.* **14**, 1263–1273. (doi:10.1111/j.1365-294X.2005.02481.x)

Nei, M. 1987 *Molecular evolutionary genetics*. New York, NY: Columbia University Press.

Nei, M. & Tajima, F. 1981 Genetic drift and estimation of effective population size. *Genetics* **98**, 625–640.

Nunney, L. & Elam, D. R. 1994 Estimating the effective population-size of conserved populations. *Conserv. Biol.* **8**, 175–184. (doi:10.1046/j.1523-1739.1994.08010175.x)

Osborne, M. J., Benavides, M. A. & Turner, T. F. 2005 Genetic heterogeneity among pelagic egg samples and variance in reproductive success in an endangered freshwater fish, *Hybognathus amarus*. *Env. Biol. Fish.* **73**, 463–472. (doi:10.1007/s10641-005-3215-3)

Pearse, D. E. & Crandall, K. A. 2004 Beyond F_{ST}: analysis of population genetic data for conservation. *Conserv. Genet.* **5**, 585–602. (doi:10.1007/s10592-003-1863-4)

Platania, S. P. & Altenbach, C. S. 1998 Reproductive strategies and egg types of seven Rio Grande Basin cyprinids. *Copeia* **1998**, 559–569. (doi:10.2307/1447786)

Poulsen, N. A., Nielsen, E. E., Schierup, M. H., Loeschcke, V. & Gronkjaer, P. 2006 Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Mol. Ecol.* **15**, 321–331. (doi:10.1111/j.1365-294X.2005.02777.x)

Rowe, G. & Beebee, T. J. C. 2004 Reconciling genetic and demographic estimators of effective population size in the anuran amphibian *Bufo calamita*. *Conserv. Genet.* **5**, 287–298. (doi:10.1023/B:COGE.0000031145.06421.d3)

Sheppard, P. R., Comrie, A. C., Packin, G. D., Angersbach, K. & Hughes, M. K. 2002 The climate of the US Southwest. *Climate Res.* **21**, 219–238.

Sublette, J. E., Hatch, M. D. & Sublette, M. A. 1990 *Fishes of New Mexico*. Albuquerque, NM: University New Mexico Press.

Sunnucks, P., Wilson, A. C. C., Beheregaray, L. B., Zenger, K., French, J. & Taylor, A. C. 2000 SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Mol. Ecol.* **9**, 1699–1710. (doi:10.1046/j.1365-294x.2000.01084.x)

Taylor, C. M. & Miller, R. J. 1990 Reproductive ecology and population structure of the plains minnow,

Hybognathus placitus (Pisces, Cyprinidae), in central Oklahoma. *Am. Midl. Nat.* **123**, 32–39. (doi:10.2307/2425757)

Turner, T. F., Dowling, T. E., Broughton, R. E. & Gold, J. R. 2004 Variable microsatellite markers amplify across divergent lineages of cyprinid fishes (subfamily Leusicinae). *Conserv. Genet.* **5**, 279–281. (doi:10.1023/B:COGE.0000029998.11426.ab)

Turner, T. F., Richardson, L. R. & Gold, J. R. 1999 Temporal genetic variation of mtDNA and effective female population size of red drum in the northern Gulf of Mexico. *Mol. Ecol.* **8**, 1223–1230. (doi:10.1046/j.1365-294x.1999.00662.x)

Turner, T. F., Wares, J. P. & Gold, J. R. 2002 Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics* **162**, 1329–1339.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. 2004 Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**, 535–538. (doi:10.1111/j.1471-8286.2004.00684.x)

Vucetich, J. A., Waite, T. A. & Nunney, L. 1997 Fluctuating population size and the ratio of effective to census population size. *Evolution* **51**, 2017–2021. (doi:10.2307/2411022)

Wang, J. L. 2001 A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet. Res.* **78**, 243–257.

Wang, J. L. & Caballero, A. 1999 Developments in predicting the effective size of subdivided populations. *Heredity* **82**, 212–226. (doi:10.1038/sj.hdy.6884670)

Waples, R. S. 1989 A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**, 379–391.

Waples, R. S. 2002*a* Definition and estimation of effective population size in the conservation of endangered species. In *Population viability analysis* (ed. S. R. Bessinger & D. R. McCullough), pp. 147–168. Chicago, IL: University Chicago Press.

Waples, R. S. 2002*b* Evaluating the effect of stage specific survivorship on the N_e/N ratio. *Mol. Ecol.* **11**, 1029–1037. (doi:10.1046/j.1365-294X.2002.01504.x)

Waples, R. S. 2005 Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Mol. Ecol.* **14**, 3335–3352. (doi:10.1111/j.1365-294X.2005.02673.x)

Weir, B. S. & Cockerham, C. C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)

Welker, T. L. & Scarnecchia, D. L. 2004 Habitat use and population structure of four native minnows (family Cyprinidae) in the upper Missouri and lower Yellowstone rivers, North Dakota. *Ecol. Fresh. Fish* **13**, 8–22. (doi:10.1111/j.0906-6691.2004.00036.x)