MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 2832–2844 doi: 10.1111/j.1365-294X.2010.04695.x

Genetic effective size, N_e, tracks density in a small freshwater cyprinid, Pecos bluntnose shiner (*Notropis simus pecosensis*)

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Abstract

Genetic monitoring tracks changes in measures of diversity including allelic richness, heterozygosity and genetic effective size over time, and has emerged as an important tool for understanding evolutionary consequences of population management. One proposed application of genetic monitoring has been to estimate abundance and its trajectory through time. Here, genetic monitoring was conducted across five consecutive years for the Pecos bluntnose shiner, a federally threatened minnow. Temporal changes in allele frequencies at seven microsatellite DNA loci were used to estimate variance effective size (N_{eV}) across adjacent years in the time series. Likewise, effective size was computed using the linkage disequilibrium method (N_{eD}) for each sample. Estimates of N_e were then compared to estimates of adult fish density obtained from traditional demographic monitoring. For Pecos bluntnose shiner, density (catch-per-unit-effort), N_{eV} and N_{eD} were positively associated across this time series. Results for Pecos bluntnose shiner were compared to a related and ecologically similar species, the Rio Grande silvery minnow. In this species, density and N_{eV} were negatively associated, which suggested decoupling of abundance and effective size trajectories. Conversely, density and N_{eD} were positively associated. For Rio Grande silvery minnow, discrepancies among estimates of N_e and their relationships with adult fish density could be related to effects of high variance in reproductive success in the wild and/or effects of supplementation of the wild population with captive-bred and reared fish. The efficacy of N_e as a predictor of density and abundance may depend on intrinsic population dynamics of the species and how these dynamics are influenced by the landscape features, management protocols and other factors.

Keywords: effective size, genetic monitoring

Received 23 December 2009; revision received 29 April 2010; accepted 30 April 2010

Introduction

Genetic monitoring is defined as tracking population genetic parameters including allelic richness, heterozygosity and genetic effective size (N_e) over time, and has been advocated as a means of assessing conservation status of species (Schwartz *et al*. 2007; Palstra & Ruzzante 2008). Knowledge of a population's census size (N_c) is also important for management as it is widely recognized that small populations will exhibit lower levels of genetic diversity than large populations because of increased genetic drift and/or inbreeding (Hartl & Clark 1989). However, it is not N_c but N_e that determines the rate of loss of variation each generation. Effective size is generally lower than census size in wild

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populations but there is not a consistent relationship between N_c and N_e. For example, in terrestrial vertebrates, the ratio of N_e to N_c may be closer to one while in marine species such as oysters the ratio maybe as low as 10^{-5} (Hedgecock 1994; Frankham 1995; Hedrick 2005). Frankham (1995) and Vucetich *et al.* (1997) suggested that fluctuation in census size was the largest contributor to the reduction of effective to census size ratios on both theoretical and empirical grounds, whilst Storz *et al.* (2001) implicated variance in reproductive success as a pivotal factor based on results of paternity analysis. If the former is correct, then genetic monitoring may be an efficient and independent means of estimating abundance of exploited populations of conservation and/or commercial interest (Ovenden *et al.* 2007; Waples *et al.* 2008; Carson *et al.* 2009).

Recently, monitoring changes in N_e over time has been suggested as an alternative to traditional population monitoring (Schwartz *et al.* 2007; Schwartz & Monfort 2008). In some species (like small-bodied fish) it may be extremely difficult, time consuming and expensive to accurately determine N_c (Simonson & Lyons 1995). Moreover, abundance can change by orders of magnitude from one year to the next in some short-lived species. In these cases it may seem appealing to replace demographic monitoring with genetic monitoring and to use genetic effective size as a measure of a population's conservation status. However, many factors affect the relationship between N_e and N_c so species' abundance may be correlated with effective size in some cases but not in others. For example, Palstra *et al.* (2009) and Saarinen *et al.* (2010) recently showed that density-dependent effects such as genetic compensation may cause decoupling of N_e from N_c. Genetic compensation occurs when reduction in the effective number of breeders (N_b) is buffered by reduced competition for mates or spawning sites when N_c is small. The converse of this scenario is depensation (or the Allee effect) in which there is a reduction in population growth rate coupled with reduced population size (Shelton & Healy 1999; Frank & Brickman 2000). This can ultimately causes a decline in the ratio of N_e to N_c (e.g. Rowe *et al.* 2004).

In this article, we employed a genetic monitoring approach to study the cyprinid fish, Pecos bluntnose shiner (*Notropis simus pecosensis*), to assess its conservation status in terms of several measures of genetic diversity. In addition, we examined whether temporal method and linkage disequilibrium estimates of N_e were correlated with estimates of density of Pecos bluntnose shiner over five consecutive years. The temporal method requires at least two samples over which the change in allele frequencies is used to estimate N_e (Nei & Tajima 1981) whilst the linkage disequilibrium method is a single-sample estimator (Hill 1981).

The Pecos bluntnose shiner is a subspecific form of the bluntnose shiner (Chernoff *et al.* 1982). The other subspecies, the Rio Grande bluntnose shiner (*Notropis simus simus*), is presumed extinct (Bestgen & Platania 1990). In the past 100 or more years, distribution and abundance of Pecos bluntnose shiner have declined considerably following habitat changes facilitated by installation of dams and alteration of the natural flow regime, changing land use, and excessive groundwater pumping (Hatch *et al.* 1985; Hoagstrom 2003; Hoagstrom *et al.* 2008 a,b). Consequently, the Pecos bluntnose shiner was listed under the Endangered Species Act in 1987 (U.S. Department of the Interior, Federal Register 1987). The adult population of Pecos bluntnose shiner is now restricted to a 150-km segment (juveniles are found downstream of this stretch) prone to river intermittency that is nested within an undammed, 333 km stretch of the Pecos River extending from downstream of Fort Summer Diversion Dam to above Brantley Reservoir, New Mexico (Hatch *et al.* 1985; Propst 1999; Hoagstrom *et al.* 2008b). Preliminary population monitoring indicated that mean annual densities and percent species composition were low in 1992 due to streamflow intermittence that occurred during between 1989 and 1991 (U.S. Fish and Wildlife Service 1992; Robertson 1997). Densities increased gradually between 1991 and 2002; a time of perennial streamflow and relatively high and stable mean discharge (Hoagstrom *et al.* 2008a). The Pecos bluntnose shiner population declined dramatically between 2002 and 2005 reaching very low densities by 2005 coinciding with three consecutive years of river intermittence (Hoagstrom *et al.* 2008b). We compared results for Pecos bluntnose shiner to a similar dataset obtained for Rio Grande silvery minnow (*Hybognathus amarus*). Like Pecos bluntnose shiner, Rio Grande silvery minnow is federally listed (U.S. Department of the Interior 1994), and is a closely related and ecologically similar cyprinid species. It differs in that it occupies a 280 km, highly fragmented stretch of the mainstem Rio Grande where the longest reach of uninterrupted river is 90 km. Rio Grande silvery minnow is heavily managed and the wild population is supplemented with individuals bred and reared in captivity (Osborne *et al.* 2006). Previous genetic studies of this species show that pelagic early life history and river fragmentation interact to decrease variance N_e values that are three orders of magnitude below N_c (Alò & Turner 2005; Osborne *et al.* 2005; Turner *et al.* 2006).

Our goal in this study was to estimate genetic effective size over a time series of 5 years (roughly equal to generations) for each species, and then compare N_e to estimates of density obtained from population monitoring studies. We predicted that the magnitude of N_e would differ among these species, but that values of

N_e would be significantly and positively related to density for both species when genetic and demographic survey data were compared across similar time series.

Materials and methods

Demographic monitoring

Fish collections used to estimate density (as measured by catch-per unit-effort [CPUE]) were made as described in Hoagstrom *et al.* (2008a,b) for Pecos bluntnose shiner and in Dudley *et al.* (2003) for Rio Grande silvery minnow. Density is the total number of fish caught divided by amount of effort (measured as the total area covered by a seine net). The use of density to monitor spatial and temporal trends in fish abundance in shallow river habitats is widely accepted in fisheries science and has been shown by both experimental and statistical analysis to be a valid means of estimating abundance (Richards & Schnute 1986). Pecos bluntnose shiner spawns from May through August (Hatch *et al.* 1985) which is more protracted than Rio Grande silvery minnow. Rather, Rio Grande silvery minnow spawn during the months of May and June with the majority of spawning occurring over a few days when flows are elevated due to snow-melt runoff and rainstorm events (Platania & Dudley 2006). To estimate density of adult fishes, mean catch rates and standard errors across collection localities were calculated for the period (January to April) preceding spawning. The overwhelming majority of sampled adult fish were age 1 (Hoagstrom *et al.* 2008b), and so reflect a single cohort. To enable comparison with temporal method estimates of N_e, mean CPUE and standard errors across collection localities between consecutive years were also calculated. In essence the same survey methods used for each species and hence estimates are comparable. For Pecos bluntnose shiner CPUE data were available for 2001–2009 and from 1999 to 2008 for Rio Grande silvery minnow.

Genetic sampling

Between 2004 and 2009, fin clips of adult Pecos bluntnose shiners were collected annually by seining at 14 sites on the Pecos River. These sites covered the present-day distribution of the species that extends from Fort Sumner diversion dam to Brantley Reservoir, New Mexico. The data set also included fin clips from fish collected in 2002 that were sampled from three sites (a subset of above) and held in captivity to mitigate effects of a severe drying event that occurred that year.

Fishes were anaesthetized in MS222 and a small piece of the caudal fin was removed and stored in 95% ethanol. Fishes were then placed in untreated water at the site of capture to recover prior to release. Fin clips of Rio Grande silvery minnow were collected in the same manner between 1999 and 2009 from throughout the occupied range in the Rio Grande, New Mexico. Further details on sampling localities can be found in Osborne *et al.* (2005).

Molecular methods

Genomic DNA was isolated from air-dried fin clips using standard proteinase-K digestion and phenol–chloroform methods (Hillis *et al.* 1996). Genotype data from 11 microsatellite loci *Lco1*, *Lco6*, and *Lco3* (Turner *et al.* 2004), *Ca6* and *Ca8* (Dimsoski *et al.* 2000), *Ppro*118 and *Ppro*126 (Bessert & Ortí 2003), *Nme208*, *Nme174*, *Nme232* and *Nme93* (Gold *et al.* 2004) were obtained for all Pecos bluntnose shiner individuals. Microsatellite loci were amplified via multiplex PCR (1 × PCR buffer, 2 mM $MgCl_2$, 125 μM dNTPS, 0.4 μM of each primer and 0.375 units of TAQ polymerase) with the following cycling conditions: one denaturation cycle of 90 °C for 3 min, 30 cycles of 90 °C for 20 s, 49 °C for 20 s (*Lco1* and *Ca6*; *Lco3* and *Lco6*; and *Ca8*) or 58 °C (*Nme174*; *Nme232* and *Nme93*) or 60 °C (*Ppro118* and *Ppro126*; *Nme208*) and 72 °C for 30 s followed by a final extension step of 72 °C for 30 min. PCR product (1 μL) was mixed with 10 μL of formamide and 0.3 μL of size standard (ABI HD400 or ROX350) and denatured at 90 °C for 5 min. All samples were run on an ABI3100 automated DNA sequencer and analysed using GeneMapper software (Applied Biosystems).

To estimate genetic effective size of Rio Grande silvery minnow genotype data were obtained from nine microsatellite loci: *Lco1*, *Lco3*, *Lco6*, *Lco7*, *Lco8* (Turner *et al.* 2004) and *Ca6* and *Ca8* (Dimsoski *et al.* 2000) and *Ppro118* and *Ppro126* (Bessert & Ortí 2003) using methods outlined in Turner *et al.* (2006), Alò & Turner (2005), Moyer *et al.* (2005). Microsatellite genotypes were characterized with an automated sequencer (ABI 3710, Applied Bioscience) and GeneMapper software (Applied Bioscience).

Data analysis

Genetic diversity

Microsatellite allele frequencies and descriptive statistics, including allelic richness (A_R), average inbreeding coefficients (F_{IS}) and Nei's (1987) unbiased gene diversity (H_e), were obtained using FSTAT v. 2.9.3.1 (Goudet 1995). Allelic richness was calculated using the methods described Petit *et al.* (1998). This method allows the number of alleles to be compared among populations somewhat independently of sample size (Leberg 1992, 2002) and is based on the smallest number of

individuals typed for any locus. FSTAT was also used to conduct global tests of linkage disequilibrium among all pairs of loci and to test for departures from Hardy–Weinberg equilibrium (HWE). The computer program MICROCHECKER v.2.2.1 (Van Oosterhout *et al.* 2004) was used to investigate the possible cause of deviations from HWE, including misscoring due to stuttering, presence of null alleles and large allele dropout.

Contemporary genetic effective population size

The temporal method (Pollack 1983; Waples 1989; Wang & Whitlock 2003) was used to estimate variance effective population size (N_{eV}) from samples collected in 2002 and 2004–2009. This method relates N_{eV} to the change in allele frequencies between two temporally spaced samples. For Pecos bluntnose shiner, four microsatellites (*Lco6*, *Ca8*, *Nme208*, and *Nme174*) that were consistently out of HWE and hence had high values of F_{IS}, were not included in estimation of N_e. For Rio Grande silvery minnow, *Lco8* was excluded for the same reason. Estimates of N_{eV} and 95% confidence intervals were obtained using the method of Waples (1989) implemented in the program NeEstimator (Peel *et al.* 2004). In addition, N_{eV} (and 95% CIs) was estimated using the pseudo-maximum-likelihood method implemented in the program MLNE v.2.3 (Wang 2001).

Both fishes studied here are essentially annual, but a small proportion of individuals in the population may live longer. To account for potentially overlapping generations, estimates of N_e were obtained using the model described in Jorde & Ryman (1995, 1996). The model requires a basic life table with information on age-specific survival rates (l_i) and birth rates (b_i). Survival rate (S) was estimated from age-structured catch data for Pecos bluntnose shiner (Hoagstrom *et al.* 2008b; U.S. FWS, unpublished). Age-specific survivorship, l_i, is equal to S^{i-1} where $l_0 = 1$. Average reproductive contribution was estimated as modal body length at age i raised to the third power (Charnov *et al.* 1999). This value was multiplied by l_i to obtain the proportional contribution of each age class to the offspring pool (p_i) and then p_i values were summed over k age classes. Birth rates at each age class were divided by $\sum_{i=1}^{k} p_i$ to produce a standardized birth rate (b_i), corrected to reflect a nongrowing population with stable age structure, i.e.$\sum_{i=1}^{k} l_i b_i = 1 = R_0$. We assumed that males and females had identical mortality and reproduction schedules and the sex ratio was 1:1 [sex ratios do not significantly deviate from 1:1 in Pecos bluntnose shiner or in silvery minnow (S. Platania, pers. comm.)]. Resulting (static) life tables were used to calculate a correction factor (C) for overlapping generations by using 100 iterations of eqn 5 in Jorde & Ryman (1996). The value C accounts for variance due to mortality as a cohort passes from 1-year class to the next and for genetic covariance among cohorts (because individuals from multiple age classes are the parents of a given cohort). The mean generation length in years (G) was calculated using eqn 10 in Jorde & Ryman (1996). A previously published value of $C/G = 1.27$ (Turner *et al.* 2006) was applied in Rio Grande silvery minnow.

The linkage disequilibrium method (Hill 1981) was also used to estimate N_{eD} from microsatellite DNA data for each of the samples using the program LDNE (Waples & Do 2008). The LDNE method estimates the effective number of breeders contributing to a sample. This program implements a correction factor to account for bias that may occur when the sample size is less than the real (unknown) effective size (England *et al.* 2006). Allele frequencies that approach one or zero can affect the value of N_{eD} (Waples 2006). For this reason LDNE calculates estimates after excluding all alleles with frequencies of less than a specified critical value. Here we used $P_{crit} = 0.02$ as suggested where there the number of individuals sampled is greater than 25 (Waples & Do 2009). This value of P_{crit} generally provides a good balance between precision and bias (Waples & Do 2009). Upper- and lower-bound 95% confidence intervals for N_{eD} were calculated using the jackknife approach implemented in LDNE.

Spearman rank correlation analysis was used to explore the relationship of estimates of N_{eV}, N_{eD} and adult fish density. Correlation analysis was conducted using SPSS version 14.0. CPUE estimates in a single year were compared to pairwise temporal N_{eV} estimates as Waples (2005) showed that variance N_e is proportional to census size (and, by extension, density) of the parental generation when generations do not overlap (i.e. discrete generations). To account for weakly overlapping generations observed in Pecos bluntnose shiner and Rio Grande silvery minnow we also compared CPUE averaged by sites across consecutive years to pairwise temporal N_e estimates. Correlations were performed separately for variance N_e and mean density by site computed for sample years x and y (overlapping generations). For all correlation analysis, N_e estimates of infinity were excluded. Spearman rank correlation analysis was also conducted between N_{eD} estimates and density (within years).

Results

Pecos bluntnose shiner genetic diversity—microsatellites

Genotype data from 11 microsatellite DNA loci were obtained for 1361 individuals spanning 7 years (five of these were consecutive). All microsatellites exhibited moderate to high polymorphism (Table 1), with locus

Table 1 Summary of diversity statistics for microsatellite loci for Pecos bluntnose shiner

Microsatellite Summary Statistics	2002	2004	2005	2006	2007	2008	2009
N	107	172	22	139	338	252	331
H_e	0.842	0.849	0.849	0.859	0.843	0.843	0.854
H_O	0.670	0.645	0.657	0.694	0.679	0.664	0.644
A_R	20.699	21.313	–	22.557	21.753	21.312	21.739
F_{IS}	0.204	0.241	0.231	0.192	0.195	0.212	0.250

N, number of individuals; A_R, allelic richness; H_e, Nei's unbiased gene diversity; H_O, observed heterozygosity, F_{IS}, average inbreeding coefficient.

Lco1 the most polymorphic with 47 alleles detected across all samples (Appendix 1). There were 47 deviations (from a total of 77 tests) from Hardy–Weinberg equilibrium after standard Bonferroni correction (Sokal & Rohlf 1981) for multiple comparisons. These were explained by heterozygote deficiency. Analysis with MICROCHECKER indicated that null alleles were the most likely cause of heterozygote deficiency. There was one instance of significant linkage disequilibrium (after Bonferroni correction) between locus *Ppro118* and locus *Nme232*. Unbiased gene diversity and allelic richness were similar across years, however, heterozygosity decreased slightly in 2009.

Contemporary effective population size—microsatellites

Microsatellite allele frequencies were used to estimate contemporary N_{eV} for Pecos bluntnose shiner for 21 pairwise temporal comparisons. Generation time (G) was estimated at 1.36 and a correction factor of 2.145 was obtained, hence C/G was 1.577. Corrected estimates of N_{eV} from adjacent cohorts reveal an increase in effective size from 116 (2005–2006) to 214 between 2007 and 2008 and to 512 for 2008–2009 (Table 2, Fig. 1). Estimates from MLNE showed the same trend of increasing N_{eV} over recent years. Over the entire sampling period, effective size estimates ranged from 74.8 (95% confidence intervals 44–146) to 1004 for the 2004 to 2006 comparison (95% CI 343–∞). Estimates of N_{eV} calculated by MLNE estimates were generally larger and ranged from 99 (66.5–176) for 2002 to 2005 comparison to 1413 (95% CI 480–∞) for the 2004 to 2006 comparison.

When all loci were included, the linkage disequilibrium method gave N_{eD} that ranged from 3958 (894.2 to infinity) to infinity (2002–2006) and indicated an increase in N_{eD} between 2007 (11 275) and 2009 (86 638) (Fig. 3b). Restricting the analysis to only seven loci resulted in estimates of infinity for all years except for

Table 2 Temporal estimates of N_e estimated from microsatellite loci and associated 95% confidence intervals Pecos bluntnose shiner. The number of years separating sampling periods are given. Asterisks indicate estimates corrected for overlapping generations

Years	Temporal (comparison)	Moments N_e	−95%	95%	ML N_e	−95%	95%
7	2002–2009	300.5	214.9	421.3	462.9	356.0	617.5
6	2002–2008	296.4	205.6	433.5	383.3	387.9	526.3
5	2002–2007	358.0	239.4	556.2	536.4	384.6	794.5
5	2004–2009	415.0	290.8	601.8	539.8	405.0	748.5
4	2002–2006	208.4	139.0	324.8	310.2	221.1	465.0
4	2004–2008	475.8	309.7	778.0	480.0	341.7	722.4
4	2005–2009	196.5	102.8	573.1	316.8	215.1	531.2
3	2002–2005	74.8	44.1	146.2	98.8	66.5	175.6
3	2004–2007	356.2	236.8	562.1	471.4	338.1	705.0
3	2005–2008	134.7	71.1	371.4	197.9	137.2	320.5
3	2006–2009	340.0	217.7	574.4	434.8	307.8	668.5
2	2002–2004	94.3	65.3	138.4	163.3	125.5	224.1
2	2004–2006	1004.3	342.3	∞	1412.8	480.4	∞
2	2005–2007	105.9	53.7	350.6	301.0	183.7	700.6
2	2006–2008	256.0	155.6	478.6	275.5	193.4	435.2
2	2007–2009	159.6	116.1	219.3	262.2	209.3	338.1
1	2004–2005*	484.4	83.4	∞	1003.1	229.2	∞
1	2005–2006*	115.9	49.1	4484.1	321.9	171.1	1856.8
1	2006–2007*	196.0	123.2	343.7	370.3	265.9	584.7
1	2007–2008*	213.9	142.9	334.4	386.1	283.3	581.2
1	2008–2009*	512.0	278.1	1304.8	1253.9	630.6	9708.1

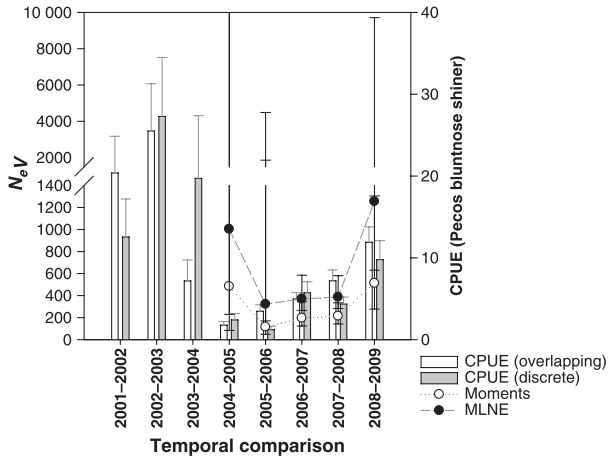

Fig. 1 Moments-based and MLNE effective size estimates (N_{eV}) and density (CPUE) assuming discrete and overlapping generations for Pecos bluntnose shiner. 95% confidence intervals (black lines) are shown for effective size estimates. Standard errors are shown for CPUE data (grey lines). For discrete generations, CPUE is calculated from the first year of the temporal comparisons.

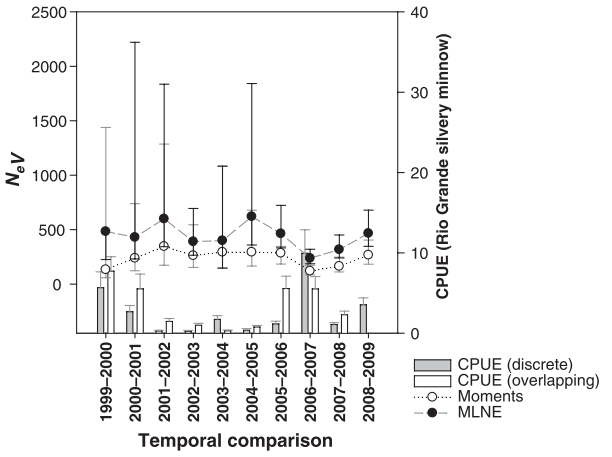

Fig. 2 Moments-based and MLNE effective size estimates (N_{eV}) and density (CPUE) assuming discrete and overlapping generations for Rio Grande silvery minnow. Standard errors are shown for CPUE data (grey lines). 95% confidence intervals are shown for MLNE (black lines) and moments (grey lines) effective size estimates. For discrete generations, CPUE is calculated from the first year of the temporal comparisons.

2007 and 2009. In all cases N_{eD} estimates were two orders of magnitude greater than N_{eV} estimates regardless of the method used. Lower bound CIs gave estimates of N_{eD} ranged from 87 (2005) to 1905 (2008) for seven loci and from 894 to 2388 when data from 11 loci was considered.

Effective population size and density

Pecos bluntnose shiner density was highest in 2002 with 27.2811 fish per 100 m^2 in the first trimester but declined dramatically in 2005 to 1.2196 fish per 100 m^2. Catch rates increased gradually from 2005 with a density of 14.0385 fish per 100 m^2 in 2009. If we assume overlapping generations, Spearman rank correlation of the remaining samples showed positive but nonsignificant relationships between the mean (across pairwise temporal samples) density and moments-based and MLNE estimates of N_e (Rho = 1.0 P = 0.083; Rho = 1.0, P = 0.083) (Fig. 1). Correlation coefficients were also positive but not significant for comparisons between mean density (within years) and moments-based and MLNE estimates of N_e (Rho = 0.80, P = 0.33; Rho = 0.8, P = 0.33). There was also a positive correlation between N_{eD} and density (Rho = 1 P = 0.083) (Fig. 3a).

Comparative analysis with Rio Grande silvery minnow

For Rio Grande silvery minnow genotype data were obtained for nine microsatellites that were assayed for temporal samples collected in 1987 and annually from 1999 to 2009. Four microsatellites (*Lco3*, *Lco6*, *Ca6* and *Ppro126*) were in HWE in all temporal samples. *Lco1* and *Lco6* were out of HWE in four and six samples respectively whilst *Ca8* and *Ppro118* were out of HWE in nine samples. *Lco8* was not in HWE in any of the temporal samples. There was no evidence of linkage disequilibrium amongst loci. Estimates of N_e for adjacent cohorts ranged from 120 (2005–2006) to 346 (2001–2002) whilst MLNE estimates were consistently larger and ranged from 238 (2006–2007) to 619 (2004–2005) (Fig. 2). The linkage disequilibrium method produced estimates of infinity for 1987, 1999–2000 and 2007 regardless of whether eight or nine microsatellite loci were used in the analysis. The lowest N_{eD} estimates were observed in the 2004 cohort N_{eD} = 540 (eight loci) and 595 (nine loci) and the highest in 2005 (N_{eD} = 3929) or 2008 (N_{eD} = 4434) depending on whether eight or nine loci were used.

Mean density ranged from a low of 0.236 (SE = 0.103) fish per 100 m^2 in 2002 to a high of 9.948 (SE = 2.918) fish per 100 m^2 in 2006. Mean density (across years) was negatively associated with moments estimates of N_{eV} (Rho = −0.615, P = 0.067) as was mean density (within years) (Rho = −0.588, P = 0.066). There was no association between MLNE estimates and mean density calculated within or across years (Rho = −0.000, P = 0.983; Rho = −0.200, P = 0.559) (Fig. 3b). Estimates of N_{eD} and density were positively associated (Rho = 0.679, P = 0.074).

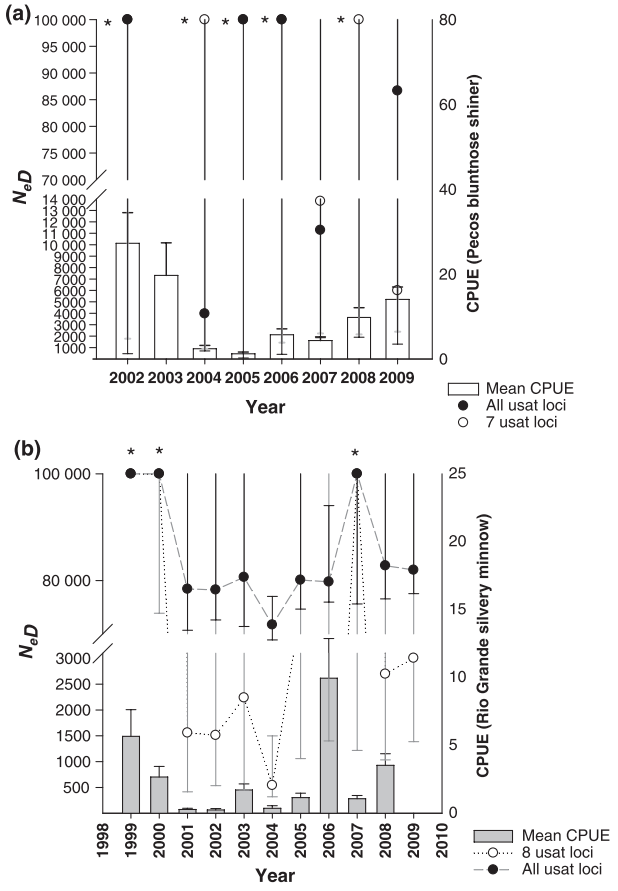

Fig. 3 N_{eD} and density (CPUE, fish per 100 m^2) for (a) Pecos bluntnose shiner (PBS) and (b) Rio Grande silvery minnow (RGSM). 95% confidence intervals are shown for N_{eD} estimates and standard errors are shown for CPUE data. Asterisks by N_{eD} points indicate estimates of infinity. For PBS and RGSM, SE bars for CPUE are shown by thick black lines. For all microsatellite loci for PBS 95% CIs are shown by thick grey lines and for seven microsatellite loci, 95% CIs are shown by thin black lines. For eight loci for RGSM 95% CIs are shown by grey lines and for all microsatellite loci 95% CIs are shown by black lines.

Discussion

One goal of genetic monitoring is to infer changes in abundance from metrics such as genetic diversity, allele frequencies and genetic effective population size (Schwartz *et al.* 2007). Genetic monitoring is potentially advantageous as it can provide information that is relevant at both contemporary and evolutionary time scales and in many cases may be more cost effective, sensitive, and reliable than population monitoring via mark-recapture techniques and other methods (Tallmon *et al.* 2010). In principle, contemporary estimates of N_e (e.g. those provided by the temporal [N_{eV}] and linkage disequilibrium [N_{eD}] methods) should be positively correlated with abundance and density across sequential measurements in a time series (e.g. Waples 2005; Tallmon *et al.* 2010; Waples *et al.* 2010). A positive trend between these measures was observed for Pecos bluntnose shiner, as expected. However, for Rio Grande silvery minnow, estimates of N_{eV} and density were negatively associated, whereas estimates of N_{eD} and density were positively associated. As we will discuss below, conflicting results are potentially explained by interspecific differences in population management (e.g. supplemental stocking for Rio Grande silvery minnow) and the degree of fragmentation of wild populations that differentially affects mortality between species.

Baseline estimates of genetic diversity

Over the course of the study, Pecos bluntnose shiner experienced dramatic declines in density, but no concomitant losses of genetic diversity (allelic richness and heterozygosity). Rather, diversity remained stable from 2002 to 2009. Levels of microsatellite diversity (average across years H_e = 0.850) were comparable to that of an abundant, co-occurring (but introduced) cyprinid, the plains minnow (*Hybognathus placitus*). Conversely, Rio Grande silvery minnow experienced detectable reductions in gene diversity and microsatellite heterozygosity over the study period (Turner *et al.* 2006).

Interspecific differences in the trajectory of heterozygosity and gene diversity may relate to effects of habitat fragmentation by dams. Fragmentation is severe in the Rio Grande study area, but not in the Pecos River. In the Pecos, the absence of barriers (dams) within the range of Pecos bluntnose shiner may allow fish to persist in high quality, wetted refugia during periods of river intermittency, with enough individuals surviving harsh conditions to repopulate rewetted areas without significant losses of diversity. Furthermore, groundwater recharge maintains wetted refugia in the upper critical habitat of Pecos bluntnose shiner (Hatch *et al.* 1985). Presumably, Rio Grande silvery minnow also move to refugia during dry periods, but these are likely to be separated by diversion dams and are often ephemeral due to losses to ground water and/or evaporation (Scurlock 1998). Pecos River refugia appear to be better connected, larger, and more suitable for sustaining populations through periods of drought compared to those in the Rio Grande.

Mortality during early life history is also hypothesized to strongly affect diversity in these species, especially in highly fragmented environments. This is because both species produce many small, buoyant eggs that can be displaced over 100 km downstream of spawning sites (Dudley & Platania 2007). Historically, larvae of both species presumably developed in

productive nursery habitats downstream and then returned upstream as juveniles and adults. Upstream movement of Rio Grande silvery minnow is now prohibited by dams. Particle transport models and empirical data suggest that many, if not most, eggs and larvae are transported to now highly modified downstream reaches that tend to dry in summer (because of water extraction) or to a reservoir where large nonnative predators await (Dudley & Platania 2007). Genetic data suggest that reproductive output from relatively few parents is retained upstream (Alò & Turner 2005). Thus, erosion of diversity in Rio Grande silvery minnow appears to result from an interaction of habitat fragmentation, early life history that promotes downstream transport (without possibility of upstream return), and negative effects of human alteration of nursery areas in downstream reaches (Turner *et al.* 2006). Such erosion of diversity does not appear to be the case in Pecos bluntnose shiner, despite large fluctuations in adult density from year to year.

Contemporary estimates of effective size

Estimates of N_{eV} obtained from Pecos bluntnose shiner microsatellite data were concordant among two calculation methods used here, although MLNE estimates were about twice that of temporal method estimates. Jorde & Ryman (2007) showed that the MLNE method may give inflated estimates of N_e when calculated from loci with highly skewed allele frequencies. Despite this, estimates of N_{eV} from both methods and N_{eD} mirrored population trends revealed by demographic monitoring. For example, the period between 2002 and 2005 corresponded to very low population densities in Pecos bluntnose shiner and exhibited similarly low values of N_{eV} (74.8, 98.8) and N_{eD} (2005 lower CI 87, see discussion below). More recent rebounds in the population (from 2006 on) were accompanied by increases in N_{eV} and N_{eD}. Despite a strong positive trend, rank correlations of density, N_{eV} and N_{eD} were not significant at $\alpha = 0.05$ because of the relatively small number of observations in the time series.

Although the same trend was apparent for N_{eV} and N_{eD}, estimates obtained using these two methods differed by two orders of magnitude. The LDNE method produced several estimates of infinity and also produced several that were negative. Negative estimates are produced when genetic data can be explained entirely by sampling error or when true N_e is large (Waples & Do 2009). The former explanation can be invoked for 2005 which contained only 18 individuals and for this reason the 2004–2005 temporal comparison was excluded from correlation analysis. For 2002 and 2006 samples, large true N_e (in which the signature of drift is small) is likely responsible for negative estimates. Waples & Do (2009) suggested that in cases where point estimates are negative, the lower bound of confidence intervals can be used to provide plausible limits of N_{eD}. For Pecos bluntnose shiner, these lower bound estimates (894–2388, 87–1905 for 11 and seven microsatellite loci, respectively) are more in agreement with temporal method estimates.

We did not observe the expected positive relationship of density and N_{eV} in Rio Grande silvery minnow, and recent large increases in this species' density have not been accompanied by substantial increases in N_{eV}. The relationship between N_e and density for Rio Grande silvery minnow depended on the method used to calculate N_e. Specifically, moments-based estimates of N_{eV} were negatively associated with density and no relationship was observed between MLNE and density. In contrast, density and N_{eD} were positively associated as expected.

Strictly speaking, the expected positive relationship between density and contemporary estimates of effective size relies on an implicit assumption that the ratio N_e/N_c is more or less constant. However, recent studies have demonstrated two important situations where the ratio N_e/N_c changes, especially when N_c reaches smaller values. Palstra & Ruzzante (2008) reviewed evidence for genetic compensation in salmonid fishes, where lower densities yielded low variance in reproductive success among families (because of higher availability of resources), which, in turn, yielded higher values of N_e/N_c than observed at larger values of N_c. Waples *et al.* (2010) also drew attention to density-dependant effects and noted that there was a trade-off whereby there is an ecological benefit to compensation as it promotes stability and long-term viability but there is an evolutionary cost as N_e is reduced because relatively few individuals contribute disproportionately. A study of cod by Rowe *et al.* (2004) demonstrated that N_c can become so small that the population experiences depensatory dynamics (i.e. an Allee effect) where N_e/N_c takes small values again. It is possible that genetic compensation explains the retention of genetic diversity in Pecos bluntnose shiner despite relatively low densities, but it is worth noting that compensatory effects did not appear to obscure the expected positive relationship between density and N_e. It is less clear how compensation/depensation might affect values of N_e for Rio Grande silvery minnow, but we discuss a few possibilities below.

Previously, we have shown that in Rio Grande silvery minnow there is evidence of extremely large variance in reproductive success among individuals caused by the interaction of early life history and river fragmentation. This interaction inflates inter-annual variance in allele

frequencies and depresses N_e that takes values that are three orders of magnitude lower than census size (Alò & Turner 2005; Turner *et al.* 2006). We hypothesized that this effect occurs through a sweepstakes mismatch process in which reproduction is poorly coordinated with resources for larval development and recruitment (Hedgecock 1994; Osborne *et al.* 2005). Spawning in Rio Grande silvery minnow is tightly tied to the spring snowmelt runoff and rainstorm events that occur from May through June (Platania & Dudley 2006). Mistiming or absence of spawning cues (such as runoff), can lead to poor reproductive effort. For example in 2006, there was virtually no spring runoff so there was little spawning by Rio Grande silvery minnow. Because the majority of Rio Grande silvery minnow die at age one (Propst 1999) many individuals will leave no offspring in their lifetime when conditions are adverse. Negative (or lack of) association of N_{eV} and adult density in Rio Grande silvery minnow suggests that downstream transport of larvae past dams to unsuitable nursery habitats is a density-negative or a density-independent process. Ironically, spawning success in Rio Grande silvery minnow is positively linked to high spring discharge (Turner *et al.* 2010), but these are exactly the conditions that facilitate downstream transport of larvae to unsuitable nursery habitats (Dudley & Platania 2007).

As noted above, the Pecos River system has features that may reduce variance in reproductive success and hence explain the strong correlation of density and N_{eV}. The current range of Pecos bluntnose shiner exceeds 300 km of continuous river habitat, allowing for greater retention of eggs and larval fish in habitats suitable for development (Dudley & Platania 2007) and the lack of fragmentation allows free movement of adult individuals throughout this range. The length of the spawning season is another key difference between these species. Pecos bluntnose shiner spawning is also cued by increases in flow such as occur during rainstorm events but this species has a more protracted reproductive period (May–August, Hatch *et al.* 1985). This may provide Pecos bluntnose shiner with greater resiliency when compared with Rio Grande silvery minnow and hence variation in lifetime reproductive success may be reduced for Pecos bluntnose shiner.

An additional factor that may explain the discrepancies of N_{eV}, N_{eD}, and their relationships with density in Rio Grande silvery minnow, is addition of captive-bred fish to the wild population. In years when there is poor spawning or recruitment of wild fish (e.g. 2006), captive-bred fish may comprise a large portion of the subsequent generation and this may cause N_e to be lower than expectations through the Ryman–Laikre effect (Ryman & Laikre 1991). Waples & Do (2008) suggested that high migration rates among weakly differentiated populations (such as between wild and hatchery samples) would provide an N_{eD} estimate that was closer to N_e of the meta-population rather than the local population (such as the wild sample) but that this effect would only be significant if migration rates were high, such as for Rio Grande silvery minnow. Since 2002, over 1.1 million adult Rio Grande silvery minnow bred and/or reared in captivity have been released to the Rio Grande, New Mexico (J. Remshardt U.S. Fish and Wildlife Service, pers. comm.).

Conclusions

Comparative genetic and demographic monitoring in these two arid land fish species indicated that the expected association of N_e and density (as a surrogate of N_c) is not always observed. Our results further suggest that correct interpretation of genetic monitoring data, especially in the context of relationship to density and abundance, requires information on focal species' biology and the landscape that the species inhabits. In this sense, demographic and genetic monitoring programs are not interchangeable, are probably most useful when done in concert, and may give incomplete information when one is used alone. For example, sole reliance on estimates of density for determination of conservation status may lead managers to believe that a population is healthy (because N_c is presumed large) when N_e could be small and expose the population to risks associated with low genetic diversity. Likewise, a species that is considered genetically healthy may be subject to environmental conditions that pose direct and immediate threat to persistence [e.g. river intermittency (Hoagstrom *et al.* 2008a,b)].

Pecos bluntnose shiner and Rio Grande silvery minnow are closely related, and ecologically similar hence differences in trajectories of N_e and density are probably not strongly related to differences in life history or ecology. Rather, extrinsic factors such as differences in the degree of habitat alteration, habitat quality, and population management probably best explain discrepancies observed species and methods used to estimate N_e. More generally, our results suggest that the efficacy of N_e as a predictor of density and abundance (e.g. Ovenden *et al.* 2007; Carson *et al.* 2009) depends on population dynamics of the species under study and upon how these dynamics are influenced by the landscape and other factors. Specific biological details on mortality schedules, the magnitude of variance in reproductive success, and knowledge of the landscape features that influence these factors may be required to fully understand the relationship of genetic and demographic parameters.

Acknowledgements

Nik Zymonas (formerly New Mexico Department of Game and Fish [NMGF]) and Bob Larson (formerly NMGF) provided vital assistance with sample collection. Rio Grande silvery minnow demographic monitoring data was collected and provided by S. Platania and R. Dudley (American Southwest Ichthyological Researchers). Funding was provided by the New Mexico Department of Game in Fish, Share with Wildlife Program. Tracy Diver, Alana Sharp and Sierra Netz provided invaluable laboratory and/or field assistance. Three anonymous reviewers provided constructive comments that improved this manuscript. Samples collected under IACUC protocol #08UNM016TR100233.

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Appendix 1

Diversity statistics by year and locus for Pecos bluntnose shiner. The 2005 sample was excluded from calculation of allelic richness due to small sample size

Locus	2002	2004	2005	2006	2007	2008	2009
Ppro118							
H_e	0.949	0.943	0.950	0.932	0.954	0.944	0.945
H_O	0.738	0.802	0.810	0.711	0.771	0.787	0.778
F_{IS}	0.223	0.150	0.151	0.237	0.192	0.167	0.176
A_R	31.077	27.442	–	29.945	31.483	29.968	29.706
Ppro126							
H_e	0.305	0.381	0.689	0.437	0.359	0.335	0.438
H_O	0.276	0.335	0.636	0.401	0.329	0.321	0.398
F_{IS}	0.096	0.121	0.078	0.083	0.083	0.040	0.092
A_R	7.894	9.992	–	11.319	9.529	7.124	9.328
Lco1							
H_e	0.957	0.959	0.960	0.961	0.961	0.961	0.960
H_O	0.709	0.769	0.727	0.788	0.761	0.722	0.668
F_{IS}	0.260	0.199	0.247	0.181	0.208	0.249	0.305
A_R	33.582	35.634	–	35.431	33.937	34.769	34.956
Ca6							
H_e	0.846	0.855	0.769	0.851	0.841	0.844	0.824
H_O	0.925	0.814	0.750	0.897	0.840	0.869	0.825
F_{IS}	−0.094	0.048	0.026	−0.055	0.001	−0.030	−0.001
A_R	8.888	9.708	–	11.908	10.466	9.956	9.365
Lco 3							
H_e	0.798	0.726	0.714	0.743	0.693	0.682	0.724
H_O	0.644	0.714	0.619	0.708	0.654	0.671	0.665
F_{IS}	0.193	0.016	0.136	0.048	0.056	0.017	0.082
A_R	12.733	11.986	–	11.782	11.050	9.910	10.771
Lco 6							
H_e	0.861	0.890	0.870	0.912	0.892	0.887	0.858
H_O	0.600	0.550	0.333	0.636	0.577	0.542	0.564
F_{IS}	0.304	0.382	0.623	0.303	0.353	0.390	0.343
A_R	24.000	23.050	–	24.694	22.778	25.333	23.452
Ca8							
H_e	0.949	0.948	0.895	0.955	0.955	0.954	0.952
H_O	0.582	0.475	0.500	0.568	0.553	0.547	0.530
F_{IS}	0.388	0.500	0.449	0.406	0.421	0.427	0.444
A_R	25.847	27.033	–	30.090	27.772	26.887	27.153

Appendix 1 (Continued)

Locus	2002	2004	2005	2006	2007	2008	2009
Nme232							
H_e	0.899	0.879	0.895	0.907	0.893	0.899	0.905
H_O	0.893	0.808	0.833	0.880	0.861	0.830	0.823
F_{IS}	0.007	0.081	0.071	0.031	0.036	0.076	0.090
A_R	16.921	18.808	–	21.379	20.002	20.884	21.089
Nme93							
H_e	0.939	0.925	0.927	0.936	0.929	0.928	0.925
H_O	0.781	0.750	0.810	0.754	0.785	0.762	0.753
F_{IS}	0.169	0.190	0.129	0.194	0.155	0.179	0.186
A_R	23.687	22.635	–	24.215	23.400	22.596	22.240
Nme208							
H_e	0.864	0.917	0.854	0.927	0.916	0.928	0.933
H_O	0.664	0.576	0.524	0.617	0.737	0.667	0.525
F_{IS}	0.233	0.373	0.392	0.336	0.195	0.282	0.437
A_R	22.410	28.630	–	27.718	27.088	25.852	28.203
Nme174							
H_e	0.892	0.916	0.848	0.903	0.892	0.926	0.927
H_O	0.573	0.509	0.682	0.689	0.606	0.614	0.556
F_{IS}	0.359	0.445	0.199	0.239	0.321	0.337	0.400
A_R	20.760	21.447	–	21.569	22.177	22.615	22.867