Estimation of Effective Population Size for the Long-Lived Darkblotched Rockfish *Sebastes crameri*

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We report the variance effective population size (N_e) in darkblotched rockfish (*Sebastes crameri*) utilizing the temporal method for overlapping generations, which requires a combination of age-specific demography and genetic information from cohorts. Following calculations of age-specific survival and reproductive success from fishery data, we genotyped a sample ($n = 1087$) comprised by 6 cohorts (from 1995 to 2000) across 7 microsatellite loci. Our N_e estimate ($\hat{N}_e$) plus 95% confidence interval was $\hat{N}_e = 9157$ [6495–12 215], showing that the breeding population number could be 3–4 orders of magnitude smaller than the census population size ($\hat{N} = 24\,376\,210$). Our estimates resemble closely those found for fishes with similar life history, suggesting that the small $\hat{N}_e/\hat{N}$ ratio for *S. crameri* is most likely explained by a combination of high variance in reproductive success among individuals, genetic structure, and demographic perturbations such as historical fishing. Because small $\hat{N}_e/\hat{N}$ ratios have been commonly associated with potential loss of genetic variation, our estimates need careful consideration in rockfish management and conservation.

Although effective population size (N_e) is a central tenet in theoretical evolutionary biology, empirical estimates of N_e have been difficult to obtain directly from natural populations because key demographic parameters are not available throughout the entire life span of individuals (Waples 2002). The so-called temporal method overcomes this limitation by indirectly assessing the variance N_e from changes in allele frequencies between discrete generations (Waples 1989). Given that N_e represents the breeding population number and determines the intensity of genetic drift in a population (Hedrick 2000), genetic estimates have become crucial information in evaluating conservation risks of natural populations (Frankham 1995). For example, loss of genetic variation, inbreeding depression, and/or greater extinction threat have been commonly associated with small N_e (Lande 1988) and small N_e/N ratios (N = census population size) (Mace and Lande 1991).

One methodological refinement of the temporal method in conservation genetics has been the combination of demographic and genetic information when generations overlap. Essentially, N_e is estimated between adjacent year classes (cohorts) and corrected for overlapping generations using age-specific survivorship and reproductive success estimates (Jorde and Ryman 1995). This approach is suitable for ecologically and economically important long-lived marine species with multiple reproductive events (iteroparity). In addition, a long generation time complicates temporal sampling of allele frequencies among discrete generations, unless historically archived samples spanning several decades are analyzed (e.g., Hauser et al. 2002).

Here we combine age-specific demography and microsatellite DNA data from cohorts of the long-lived marine darkblotched rockfish (*Sebastes crameri*) to estimate variance effective population size. Darkblotched rockfish is one deep-water representative of the speciose genus *Sebastes* in the northeastern Pacific. *Sebastes crameri* is characterized by long life span (maximum age recorded 105 years; Love et al. 2002) and late maturity (males: 5 years, females: 8 years; Nichol and Pikitch 1994). Low levels of biomass motivated the Pacific Fishery Management Council to declare darkblotched rockfish overfished (Rogers et al. 2000). Our goal was to provide estimates of N_e and N and interpret their relevance to rebuilding plans (Rogers 2003) and conservation initiatives for the recovery of heavily exploited rockfish (Berkeley, Hixon, et al. 2004).

Materials and Methods

Samples

Rockfish fin clips ($n = 1206$) came from an earlier study that analyzed the genetic structure of *S. crameri* ranging from the

coast of Washington to northern California during 2001 and 2002 (Gomez-Uchida and Banks 2005). Ages of sampled fish were determined from otoliths using the break-and-burn technique (Chilton and Beamish 1982).

Molecular Analyses

Freshly extracted DNA from each individual was screened for 7 polymorphic microsatellite loci using polymerase chain reaction (PCR) as presented in Gomez-Uchida and Banks (2005). Extra amplification cycles (e.g., 5–10 cycles) and an additive containing 20 mg/ml of bovine serum albumin were necessary when PCR fragments were weak. Details on the electrophoresis procedures and sizing of PCR fragments can be found in Gomez-Uchida and Banks (2005).

Genetic Data Analyses

Multilocus genotypes were apportioned into cohorts that matched calendar years. In order to provide a global estimate of N_e (e.g., Turner et al. 2002), we pooled genotypes across sampling locations within year classes. Only genetic information from the 6 largest cohorts (1995–2000) ($n = 1087$) was used during the rest of the analyses. These year classes were homogeneously distributed across the geographic area (Gomez-Uchida D and Banks MA, unpublished data), suggesting no confounding effects of geographic and intercohort differentiation.

Allele frequencies and general statistics (e.g., allelic richness, and expected vs. observed heterozygosity per locus) for each cohort were estimated using GENETIX version 3.3 (Belkhir et al. 2002). The null hypotheses of 1) Hardy–Weinberg equilibrium (HWE) within cohorts and 2) gametic (linkage) equilibrium among loci within cohorts were tested through exact tests implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). The probability of the joint hypothesis over multiple loci was calculated through Fisher's method implemented in GENEPOP. Departures from HWE were further investigated with MICROCHECKER (van Oosterhout et al. 2004) to exclude potential genotyping errors. Finally, selective neutrality of alleles was tested through the X^2-statistic between adjacent year classes of Lewontin and Krakauer (1973) (Jorde and Ryman 1996).

Demographic Analyses and Estimation of N_e and N

Age-specific survival rates (l_i) were estimated from annual survival (S_i) calculated for 38 age classes found in stock assessments of *S. crameri* (Rogers et al. 2000; Rogers 2003). We used the expression

$$S_i = \frac{1}{m} \sum_y^m \frac{N_{i,y}}{N_{i+1,y+1}}, \qquad (1)$$

where $N_{i,y}$ is the number of individuals at age class i in the year y. In our case, S_i corresponds to the arithmetic mean obtained from $m = 40$ years (1964–2003) of fishery data (Rogers et al. 2000; Rogers 2003) to account for annual variation in mortality rates. Assuming that the survival rate of the

Table 1. Cohort genetic statistics for *Sebastes crameri*

Cohort	1995	1996	1997	1998	1999	2000
Sample size	48	82	123	67	627	140
H_E	0.69	0.70	0.69	0.70	0.71	0.71
H_O	0.69	0.68	0.68	0.70	0.71	0.70
A_R	8.57	8.39	8.89	8.31	8.99	8.89
HWE	0.36	0.08	0.10	0.46	0.68	0.11

H_E, expected heterozygosity; H_O, observed heterozygosity; A_R, allelic richness; and HWE, exact test for deviations from HWE.

first (newborn) age class, $l_1 = 1.0$ (Jorde and Ryman 1996), then $l_{i+1} = S_i l_i$.

To obtain age-specific reproductive success (p_i), we used the mean number of progeny per male (b_i^m) and female (b_i^f) for each particular age class as "proxy" estimators. These are the proportions of sexually mature rockfish at age from Nichol and Pikitch (1994) times the mean weight at age from (Rogers et al. 2000; Rogers 2003). These variables were adjusted to result in a net reproduction rate $R_0 = \sum l_i b_i = 1$ for each sex. Finally, we derived a value of reproductive success weighing both sexes equally: $p_i = l_i (b_i^m + b_i^f)/2$ (Jorde and Ryman 1996).

An estimate of N_e ($\hat{N}_e$) was obtained using the temporal method for overlapping generations as described in Jorde and Ryman (1995). Briefly, we used the equation:

$$\hat{N}_e = \frac{C}{2G\bar{F}'}, \qquad (2)$$

where C is a correction factor obtained from l_i and p_i, G is the mean generation length, and $\bar{F}'$ is the grand mean of temporal allele frequency change (among all adjacent cohorts and over all loci) derived from Pollak (1983) and corrected for sampling variance. Equations to compute $\bar{F}'$, C, and G are found in Jorde and Ryman (1995). In addition, the 95% confidence interval (CI) for $\bar{F}'$ and $\hat{N}_e$ was calculated according to Waples (1989) assuming a chi-square distribution for F.

The estimated census population size ($\hat{N}$) corresponded to the arithmetic mean of total numbers of fish in the population between 2001 and 2002 (Rogers et al. 2000; Rogers 2003).

Results and Discussion

Cohort genetic statistics did not vary significantly among cohorts; in addition, no significant deviations from HWE were found and linkage equilibrium was met in all pairwise loci comparisons within each year class (Table 1). MICROCHECKER did not detect genotyping errors or null alleles. Lastly, the X^2-statistic of Lewontin and Krakauer (1973) suggests that changes in microsatellite allele frequencies between cohorts are the sole effect of genetic drift (Table 2).

We observed a nearly exponential decline in survival (l_i) as a function of age; on the contrary, reproductive success (p_i) peaked at 8–12 years (Figure 1). Both age-specific variables behaved according to theoretical expectations (e.g., Jorde and Ryman 1995). With these results, we then calculated the

Table 2. Probability values against the null hypothesis of selective neutrality (Lewontin and Krakauer 1973) and the estimator of allele frequency change corrected for sampling error (F') calculated between adjacent cohorts of *Sebastes crameri*. $\bar{F}'$ is the arithmetic mean over all cohort pairs

Cohort pair	1995–1996	1996–1997	1997–1998	1998–1999	1999–2000	Mean ($\bar{F}'$)
$P(X^2)$	0.64	0.14	0.52	0.35	0.30	
F'	0.0032	−0.0001	0.0014	−0.0017	0.0005	0.0006

following values: $C = 110.89$ and $G = 9.7$. An estimate of $\bar{F}'$ [95% CI] = 0.0006 [0.0005–0.0009] was obtained by averaging F' values across cohort pairs shown in Table 2. This provides an overall point estimate plus 95% CI of $\hat{N}_e = 9157$ [6495–12 215]. Based on $\hat{N} = 24\ 376\ 500$, the ratio $\hat{N}_e/\hat{N}$ could therefore vary between 0.001 and 0.0001 for darkblotched rockfish.

Wright (1931) predicted that under ideal conditions—even sex ratio, nonoverlapping generations, constant population size, panmixia, and Poisson variance in reproductive success among individuals—the ratio N_e/N should approach unity. However, $\hat{N}_e/\hat{N}$ in natural populations average 0.1 using a variety of analytical methods (Frankham 1995), clearly indicating that these ideal conditions are commonly violated in real populations. What biological phenomena can explain the strikingly large discrepancy we observe between $\hat{N}_e$ and $\hat{N}$ values in darkblotched rockfish?

First, uneven sex ratios cannot greatly explain the small ratio $\hat{N}_e/\hat{N}$ found in *S. crameri* because extensive life-history data suggest no significant departures from 1:1 sex ratios (Nichol and Pikitch 1994; Rogers et al. 2000; Rogers 2003). Second, overlapping generations certainly violate ideal conditions. However, we have employed an ad hoc approach, with stable age structure and constant population size being the basic assumptions of the model. Ages are assumed to be errorless in the theoretical model; however, Rogers (2005) provides evidence that aging errors are commonplace in stock assessments of *S. crameri*, particularly for older individuals (>20 years old). Imprecise ages are nevertheless more likely to impact the accuracy of N than $\hat{N}_e$ because 1) we

Figure 1. Survival rates (l_i) (empty squares) and rates of reproductive success (p_i) (filled triangles) as a function of age in *Sebastes crameri*.

used fish ranging from ages 1 to 7 to obtain the genetic information and 2) the correction factor (C) and mean generation length (G) depend primarily on survival and reproductive success, with both variables decaying rapidly for fish older than 12 years old (Figure 1).

Historical fishing has been demonstrated to truncate age structure and to diminish the population number of numerous groundfish stocks, including our species (Berkeley, Hixon, et al. 2004). From Jorde and Ryman (1996), it is clear that a reduction in age classes will decrease C and $\hat{N}_e$ will therefore be underestimated; they further argue that monitoring the population over time can circumvent demographic disturbances affecting the correction factor C. Long-term genetic data collections during the current stage of stock recovery are thus necessary to validate C and $\hat{N}_e$ in darkblotched rockfish.

Third, both significant spatial structure and genetic isolation by distance suggest that darkblotched rockfish is not best explained by a panmictic model (Gomez-Uchida and Banks 2005). In principle, a metapopulation with stepping-stone migration can be considered a large single panmictic population for purposes of estimating effective size if the overall genetic differentiation is small (Wang and Caballero 1999) as found in Gomez-Uchida and Banks (2005) for *S. crameri*. Although differences in size among subpopulations might potentially bias our $\hat{N}_e$ downwardly (Wang and Caballero 1999), it is unlikely that this alone could account for the large difference in magnitude observed between $\hat{N}_e$ and $\hat{N}$.

Fourth, our estimates seem to agree closely with published $\hat{N}_e$ and $\hat{N}_e/\hat{N}$ ratios for marine fishes with similar life history, including red drum (Turner et al. 2002), New Zealand snapper (Hauser et al. 2002), and Atlantic cod (Hutchinson et al. 2003). These fishes possess both high fecundity and mortality during early life stages (type III survivorship); this life strategy, when coupled with highly variable oceanographic conditions, could lead to a larger variance in reproductive success among individuals (V_k) than expected under a Poisson distribution, with the small minority of individuals ($\hat{N}_e$) replacing the entire population ($\hat{N}$) in every generation (Hedgecock 1994). Recent findings in black rockfish (*Sebastes melanops*) indicate that older females produce larvae of better quality than younger ones, hence more capable of producing competent offspring (Berkeley, Chapman, and Sogard 2004), supporting the hypothesis that V_k could vary greatly among individual female rockfish.

In conclusion, small $\hat{N}_e/\hat{N}$ for *S. crameri* is most likely explained by a combination of factors, such as high variance in reproductive success among individuals (V_k), genetic differences among subpopulations, and potential demographic disturbances such as intense fishing. Our results suggest that
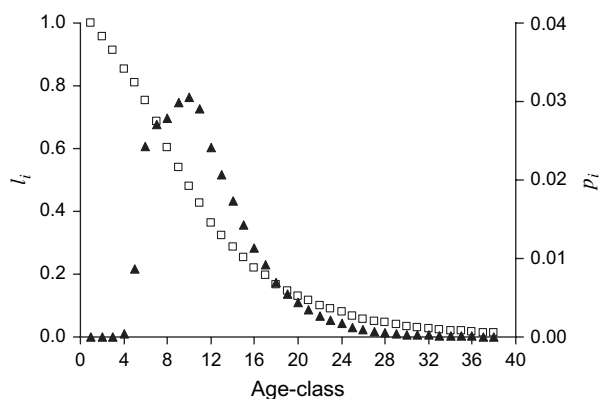
only several thousand darkblotched rockfish rather than millions in the whole population represent the breeding population number every generation. Given that small ratios $\hat{N}_e/\hat{N}$ are often found within populations of threatened species (Mace and Lande 1991), our estimates need careful consideration for management of vulnerable long-lived stocks (Musick 1999). However, further research is needed to 1) disentangle the individual effects of the aforementioned factors on N_e and 2) obtain independent estimates of key demographic parameters (e.g., Waples 2002) to validate $\tilde{N}_e/\hat{N}$ ratios across marine species. Only then will we have confidence in extending findings made here for *S. crameri* to assess long-term conservation risks of further harvest of other rockfish and marine stocks.

Acknowledgments

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