

# Genetic Effective Size Is Three Orders of Magnitude Smaller Than Adult Census Size in an Abundant, Estuarine-Dependent Marine Fish (*Sciaenops ocellatus*)

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## ABSTRACT

Using eight microsatellite loci and a variety of analytical methods, we estimated genetic effective size ( $N_e$ ) of an abundant and long-lived marine fish species, the red drum (*Sciaenops ocellatus*), in the northern Gulf of Mexico (Gulf). The ratio  $N_e/N$ , where short-term variance  $N_e$  was estimated via the temporal method from shifts in allele-frequency data over four cohorts and where  $N$  reflected a current estimate of adult census size in the northern Gulf, was  $\sim 0.001$ . In an idealized population, this ratio should approximate unity. The extraordinarily low value of  $N_e/N$  appears to arise from high variance in individual reproductive success and perhaps more importantly from variance in productivity of critical spawning and nursery habitats located in spatially discrete bays and estuaries throughout the northern Gulf. An estimate of  $N_e$  based on a coalescent approach, which measures long-term, inbreeding effective size, was four orders of magnitude lower than the estimate of current census size, suggesting that factors presently driving  $N_e/N$  to low values among red drum in the northern Gulf may have operated similarly in the past. Models that predict  $N_e/N$  exclusively from demographic and life-history features will seriously overestimate  $N_e$  if variance in reproductive success and variance in productivity among spatially discrete demes is underestimated. Our results indicate that these variances, especially variance in productivity among demes, must be large for red drum. Moreover, our study indicates that vertebrate populations with enormous adult census numbers may still be at risk relative to decline and extinction from genetic factors.

GENETIC effective population size ( $N_e$ ) is an important parameter in evolutionary biology, in part because it determines standing levels of neutral genetic diversity and in part because it determines the relative effects of genetic drift and selection on nonneutral loci. Increasingly, evolutionary and conservation biologists are interested in genetic effective size as an indicator of long-term risk of extinction from genetic factors. Populations with small  $N_e$  may suffer reduced capacity to respond to changing or novel environmental pressures, inbreeding depression, and/or accumulation of deleterious alleles (FRANKHAM 1995; HIGGINS and LYNCH 2001).

Despite its importance for understanding evolutionary processes,  $N_e$  is notoriously difficult to estimate in natural populations. However, the connection between  $N_e$  and life history, behavioral ecology, and demography is becoming better understood (CABELLERO 1994; WANG and CABELLERO 1999). WRIGHT (1931) originally envisioned the concept of genetic effective size under idealized conditions: These included panmixia, a 1:1 sex ratio, nonoverlapping generations, Poisson variance in reproductive success, and temporally stable population number. Subsequent development of theory for estimating

$N_e$  in “real” populations has emerged from evaluating effects of violations of idealized population assumptions (WRIGHT 1939; HILL 1979; CHESSER *et al.* 1993; CABELLERO 1994; WHITLOCK and BARTON 1997; NUNNEY 1999). This work has led to development and application of demographic models for predicting effective size without explicit genetic data (*e.g.*, NUNNEY and ELAM 1994) or with demographic and limited genetic data in structured populations (*e.g.*, analysis of spatial genetic variance via hierarchical *F*-statistics; see BASSET *et al.* 2001).

In many demographic models, emphasis is placed on relating  $N_e$  to estimates of adult census size ( $N$ ) to estimate the ratio  $N_e/N$ . This is convenient because an idealized population is expected to have an  $N_e/N$  ratio of one, and deviations from idealized populations are expected to have  $N_e/N$  ratios that are usually less than one. NUNNEY and ELAM (1994) and NUNNEY (1996) examined the ratio  $N_e/N$  over a wide range of life history and demographic scenarios (assuming type II survivorship, which requires uniform probability of survival across age classes, and without explicit genetic data) and found that  $N_e/N$  should rarely take values  $< 0.25$ . Their finding is potentially important because it suggests that a reasonable estimate of  $N_e$  can be obtained from census data and life history information alone, provided that good estimates of necessary data are avail-

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able. Conservation and management efforts could increasingly depend on such demographic models to evaluate long-term risks of extinction from genetic factors, especially as more species become threatened and endangered and resources for genetics-based studies become more thinly spread. Thus, it is critical to understand the limitations of demographic models for assessing risks of extinction by genetic factors.

Empirically derived  $N_e/N$  values are lower ( $N_e/N \approx 0.1$ , on average) than values obtained from demographic models (FRANKHAM 1995). This discrepancy suggests that wholesale application of demographic models may not be warranted. Differences between empirical and theoretical estimates of  $N_e/N$  are thought to arise because of difficulties in obtaining data necessary for accurate prediction of  $N_e/N$ . For example, adult census numbers over protracted periods are usually required because breeding adult census numbers in natural populations likely fluctuate over time and because such fluctuations are expected to lower (inbreeding) effective size with respect to current census size (VUCETICH *et al.* 1997). Perhaps even more difficult to obtain are reasonable estimates of variance in reproductive success among individuals (both male and female variances are required) and variance in productivity among habitats in a spatially structured population (NUNNEY 1999). Variance in reproductive success is especially important for species with very large reproductive potential but with high early mortality (*i.e.*, type III survivorship, which implies a very low probability of survival at early life stages and high survival subsequently), where successful recruitment depends on specific resources that are patchily distributed (HEDGECOCK 1994). In such species, variance in reproductive success may be of sufficient magnitude to result in values of  $N_e/N$  that are much lower than expected from demographic models that assume Poisson variance in reproductive success (*e.g.*, HEDGECOCK 1994; GAGGIOTTI and VETTER 1999; TURNER *et al.* 1999). However, there are very few empirical studies of  $N_e/N$  in species with type III survivorship, so it is not clear how well demographic models predict  $N_e/N$  in such species.

In this study, we employed a variety of genetic and demographic approaches to estimate  $N_e/N$  in an abundant marine fish species, the red drum (*Sciaenops ocellatus*). Although highly fecund and widely distributed as adults, successful recruitment in the species depends on patchily distributed estuarine habitats that vary in quality. Adult females produce numerous small offspring of presumably low quality, with the consequence that mortality in early life stages (eggs, larvae, and juveniles) is great (PATTILLO *et al.* 1997). Because the species is important economically, considerable data on population genetic structure, census size, demography, and life history have been accumulated, providing a test case for comparing various genetic and demographic models for estimating  $N_e/N$ .

## MATERIALS AND METHODS

**Ecology and life history of red drum:** Red drum (*S. ocellatus*) occur in coastal and near-shore waters of the Gulf of Mexico (Gulf) and western Atlantic Ocean (Atlantic). Successful recruitment to adult breeding populations depends on bays and estuaries that serve as nursery grounds for larvae and juveniles (MATLOCK 1987). Juveniles reside in estuaries until about age 4, when they move out of the bays and estuaries into near-shore waters (PATTILLO *et al.* 1997). Sexually mature adults can form large, migrating schools offshore, and large fish in offshore waters of the Gulf are known to move considerable distances (NICHOLS 1988; MITCHELL and HENWOOD 1999). Adults return to inlets adjacent to bays and estuaries for spawning, and eggs are swept into nursery habitats presumably by tidal action (MATLOCK 1987). Fertilization is external, with group-synchronous oocyte maturation and multiple-batch spawning (WILSON and NIELAND 1994). Maximum individual fecundity is estimated as  $3 \times 10^7$  eggs per 9- to 14-kg female (OVERSTREET 1983). There is little sexual dimorphism though females on average are slightly larger than males (WILSON and NIELAND 1994). Life span can exceed 60 years but individuals >30 years are relatively rare (WILSON and NIELAND 1994; ROSS *et al.* 1995). The species was harvested commercially until the early 1990s when commercial fishing was closed in response to declines in abundance. Red drum currently support an important recreational fishery in both the northern Gulf and Atlantic. Despite recreational fishing pressure, numbers of adults have remained essentially constant over a 10-year period. Based on tag-recapture data, NICHOLS (1988) estimated numbers of adults at 6.06 million individuals [lower- and upper-bound 90% confidence intervals (C.I.s) were 4,370,000 and 7,700,000, respectively] in the northern Gulf. Similar adult numbers were reported in a more recent tag-recapture study conducted in 1997 and 1998 (2,350,000; 90% C.I.s 1,660,000 and 3,050,000, respectively; MITCHELL and HENWOOD 1999). The harmonic mean census size across the sampling period is 3.4 million adult individuals.

Previous studies on population genetic structure of red drum have employed a variety of genetic markers including allozymes (BOHLMAYER and GOLD 1991), mitochondrial (mt)DNA (GOLD *et al.* 1999), and microsatellites (GOLD and TURNER 2002). Briefly, these studies have shown that (i) the population in the northern Gulf is distinct genetically from the population in the Atlantic and (ii) the population in the northern Gulf exhibits temporally stable and statistically significant genetic divergence among sampled bays and estuaries. Gene flow appears to conform to a modified stepping-stone model (GOLD *et al.* 2001), where individual subpopulations are centered in spatially discrete bays and estuaries but where gene exchange occurs primarily (but not exclusively) between subpopulations in adjacent bays and estuaries distributed linearly along the coastline. Gene flow does occur among subpopulations in estuaries that are not adjacent but probabilities of gene exchange decrease as a function of geographic distance (GOLD *et al.* 2001).

**Sampling for genetic analysis:** For this study, we employed microsatellite data from 967 red drum representing four year classes or cohorts (1986 to 1989) sampled from seven bays or estuaries in the northern Gulf (Figure 1). Details of sampling, aging of fish, and tissue storage are given in GOLD and TURNER (2002). Individual fish were assayed for allelic variation at eight microsatellite loci (*Soc11*, *Soc19*, *Soc35*, *Soc60*, *Soc156*, *Soc204*, *Soc243*, *Soc252*) developed specifically for red drum (TURNER *et al.* 1998). Details of DNA isolation, conditions of polymerase chain reaction (PCR) amplifications and primers, electrophoresis, and allele scoring are given in GOLD and TURNER (2002). Genotypes at each microsatellite locus in each

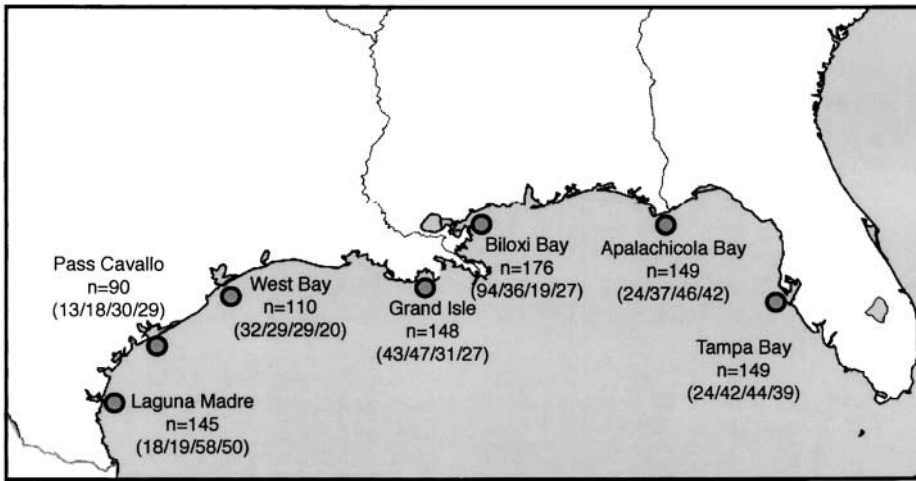


FIGURE 1.—Sampling localities and sample sizes ( $n$ ) of young-of-the-year red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico. Four cohorts (from 1986 to 1989) are represented at each locality, denoted as follows:  $n(1986)/n(1987)/n(1988)/n(1989)$ .

year-class sample were tested for departure from Hardy-Weinberg equilibrium expectations. Tests were carried out using GENEPOP version 3.1d (RAYMOND and ROUSSET 1995).

**Temporal method estimates of  $N_e$ :** We used the temporal method (NEI and TAJIMA 1981; WAPLES 1989) to evaluate standardized variance in shifts of allele frequency ( $F$ ) across cohorts (pooled across sampling localities), using the formula

$$F = \frac{1}{K} \sum_{i=1}^K \frac{(x_i - y_i)^2}{(x_i + y_i)/2 - x_i y_i}, \quad (1)$$

where  $K$  is the number of alleles, and  $x_i$  and  $y_i$  are frequencies of allele  $i$  at year  $t$  and year  $t + 1$ , respectively (NEI and TAJIMA 1981).  $F$  includes variance components due to genetic drift and to sampling the population at years  $t$  and  $t + 1$ . Variance attributed to sampling was removed to yield a corrected  $F$  value, with remaining variance attributable to genetic drift across year classes. The corrected value,  $F'$ , is calculated by

$$F' = F - \frac{1}{2S_t} - \frac{1}{2S_{t+1}}, \quad (2)$$

where  $S_t$  and  $S_{t+1}$  are sample sizes at year classes  $t$  and  $t + 1$ , respectively (WAPLES 1989), and the terms  $1/2S_t$  and  $1/2S_{t+1}$  represent the variance expected from sampling the population at both years. In addition to the moment-based, temporal-method estimator (Equation 1), we also employed the pseudo-maximum-likelihood estimator described in WANG (2001). This latter method has been shown to perform nearly as well as “full”-likelihood methods (*e.g.*, WILLIAMSON and SLATKIN 1999; ANDERSON *et al.* 2000) when estimating  $N_e$  from triallelic loci and to provide a more accurate and precise estimate of  $N_e$ , relative to estimating  $N_e$  from  $F$ -statistics, when a large number of rare alleles are present (WANG 2001). In addition, full-likelihood methods are computationally prohibitive for data sets with many alleles (E. ANDERSON, personal communication).

We pooled data by year class (cohort) across sample localities for all temporal method analyses. By pooling, we sought to provide an empirically derived estimate of  $N_e$  for the entire population of red drum in the northern Gulf under an assumption of panmixia. Our interest was to compare this value of  $N_e$  to estimates of  $N_e$  from demographic models where population structure is incorporated explicitly (WHITLOCK and BARTON 1997; NUNNEY 1999). Pooling data in this way provided more precise temporal-method estimates of  $N_e$  by increasing the total number of individuals used in the estimation and thereby decreasing the 95% confidence intervals around  $N_e$  (WAPLES 1989).

We used the analytical method developed by JORDE and RYMAN (1995, 1996) to account for effects of overlapping generations on temporal-method estimates of  $N_e$ . This correction requires information on age-specific survival rates ( $l_i$ ) and birth rates ( $b_i$ ). Two values of survivorship ( $S$ ) were used. The first was from a tag-recapture study of prereproductive fish where  $S = 0.42$  (GREEN *et al.* 1985). The second value,  $S = 0.84$ , was estimated by examining age-structure data (WILSON and NIELAND 1994) of sexually mature fish. We used these values to calculate age-specific survivorship ( $l_i = S^{i-1}$ ) for each age class  $i$ . Survivorship calculated in this way assumes an equal probability of surviving from one year class to the next and equal probability of survival of males and females.  $N_e$  was calculated using low- and high-survivorship scenarios separately to understand the effects of different age structures on estimates of  $N_e$ . Birth rate was estimated by calculating mean (wet) weight of gonads, as an indicator of relative gamete contribution, at each age class. Gonad weights (WILSON and NIELAND 1994) were averaged across males and females within each age class and this value was multiplied by  $l_i$  to obtain the proportional contribution of each age class to offspring ( $p_i$ );  $p_i$  values were then summed over  $k$  age classes. Mean gonad weights 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.*,  $\sum_{i=1}^k l_i b_i = 1 = R_0$ . Resulting life tables were used to calculate a correction factor ( $C$ ) for overlapping generations by using 100 iterations of Equation 5 in JORDE and RYMAN (1996). The value  $C$  accounts for variance due to mortality as a cohort passes from one year class to the next and for genetic covariance among cohorts (because individuals from multiple age classes are the parents of a given cohort).  $G$ , the mean generation length in years, was calculated using Equation 10 in JORDE and RYMAN (1996). These values were subsequently used to calculate (variance)  $N_e$  by

$$N_e = \frac{C}{2G\bar{F}'}, \quad (3)$$

where  $\bar{F}'$  is the grand mean of the (sampling-error corrected) standardized variance of allele frequency shifts (resulting from the moment-based approach described above).  $\bar{F}'$  was calculated as follows. First, a weighted mean  $F$  value was computed across the eight microsatellite loci by using the formula in WAPLES (1989) for each of six pairwise comparisons between all four cohorts. Weighted means were corrected for sampling error using Equation 2 and then averaged across the six pairwise comparisons to yield  $\bar{F}'$ . Under the assumption that the

largest uncertainty in  $N_c$  arises from sampling error when determining  $\bar{F}'$  (rather than estimation of  $C$  or  $G$ ), upper- and lower-bound 95% C.I.s around  $\bar{F}'$  were calculated by using a normal approximation (JORDE and RYMAN 1996). Values of  $N_c$  obtained from the pseudo-maximum-likelihood (ML) analysis were corrected for overlapping generations by taking the product of  $N_c$  (obtained from Wang's program) and  $C$  and dividing by  $G$ . Finally, we obtained the ratio  $N_c/N$  by dividing the result of Equation 3 by  $3.4 \times 10^6$ , the available estimate of  $N$  for red drum in the northern Gulf (NICHOLS 1988; MITCHELL and HENWOOD 1999).

**Coalescent method estimates of  $N_c$ :** Estimates of  $N_c$  also were derived from the genealogical structure of microsatellite alleles by using an approach based on coalescent theory (KINGMAN 1982). This estimator uses gene-tree branch lengths as descriptors of  $\Theta = 4N_c\mu$ , where  $\mu$  is the rate of substitution per generation at a genetic locus or group of loci (HUDSON 1991). For this analysis, we used the program MIGRATE (BEERLI and FELSENSTEIN 2001; BEERLI 2002), where Markov chain Monte Carlo (MCMC) sampling of gene trees is used to estimate  $\Theta$  for each sampled subpopulation as well as pairwise migration rates among subpopulations (referred to hereafter as "demes").

Sampling genealogical space for the entire data set ( $n = 967$ ) proved computationally prohibitive, so we used an iterative method to produce stable estimates of these parameters. Ten individuals were selected at random from each locality, and the resulting truncated data set ( $n = 70$ ) was analyzed by using a parallel-processing approach that employed two dual processors. Each locus was analyzed separately by using a stepwise mutation model; the MCMC search employed 10 short chains (sampling 500 gene trees) and 3 long chains (5000 gene trees), each time ignoring the first  $10^4$  steps to ensure parameter stability. This analysis was repeated twice for each locus and resulting integrated estimates of  $\Theta$  were used to initialize a more thorough analysis (20 individuals randomly selected from each population, 10 replicates each locus, using 4 long chains). In each chain, evaluating the parameters and initially assuming that they were uncorrelated generated a likelihood profile. This was followed by full-likelihood maximization, permitting an accurate estimate of the likelihood surface (BEERLI 2002). Following parallel analysis of each locus, likelihood surfaces were integrated using MIGRATE and allowing for rate variation among loci.

A geographic distance matrix also was used that permitted the coalescent analysis to account for linear distances (kilometers) among demes centered in individual bays and estuaries. We specified a stepping-stone migration matrix in MIGRATE; this provided an approximate solution of  $\Theta$  but better reflected the observed pattern of gene flow among the sample localities (GOLD *et al.* 2001). While an exact solution based on coalescent theory is not yet available for  $\Theta$  under a continuous stepping-stone model, bias in the approximation is not expected to be large when estimates of  $N_c$  are generated for each locality and summed to obtain a regional estimate (BARTON and WILSON 1995; EPPERSON 1999).

To calculate  $N_c$  from  $\Theta$ , estimates of the per gene mutation rate,  $\mu$ , are necessary. We obtained a distribution of  $\mu$  by assuming limits of  $1 \times 10^{-5}$  and  $1 \times 10^{-3}$  as the lower and upper rates of mutation at microsatellite loci. These represent empirical limits observed in a variety of organisms (JARNE and LAGODA 1996) including fishes (DE LEON *et al.* 1998). Because there is a linear relationship between  $\Theta$  and  $\mu$ , we approached the estimation of  $\mu$  by treating each population as a replicate estimate of the relative mutation rates among loci. The locus with the highest  $\Theta$  was assumed to be  $\mu = 1 \times 10^{-3}$ , with all other ( $j$ ) loci scaled according to the ratio of  $\Theta_j/\Theta_{\max}$ . This was repeated using the lowest  $\Theta$ , with  $\mu = 1 \times 10^{-5}$ , for each sample locality and scaling all other loci according to  $\Theta_j/\Theta_{\min}$ .

The range of mutation rates estimated in this way does not exceed the range of  $1 \times 10^{-5}$  to  $1 \times 10^{-3}$ , so these estimates were used to calculate lower- and upper-bound estimates of  $N_c$ .

Coalescent-based and temporal-method estimates of  $N_c$  differ in important ways. The coalescent method estimates inbreeding effective size (ORIVE 1993) and provides a long-term estimate of effective size integrated over the time to common ancestry of all alleles in the population (AVISE 2000). Conversely, the temporal method provides an estimate of variance effective size based on changes in allele frequencies over the time interval between sampling periods (WAPLES 1989), thus providing an estimate of effective size for a contemporaneous population. Variance and inbreeding effective sizes are equal for a large population of constant size (WHITLOCK and BARTON 1997), but differ under other conditions such as fluctuating effective population size (CROW and DENNISTON 1988). If effective size of a population remains stable over time, the coalescent method and the temporal method are expected to give similar values of  $N_c$ . If a population suffers a bottleneck but recovers with a burst of exponential population growth, the temporal method estimate of  $N_c$  is expected to be on the order of contemporaneous adult census size, whereas the coalescent-method estimate is expected to be on the order of the adult census size immediately following the bottleneck.

**Demographic estimates of  $N_c/N$ :** We employed the demographic model (termed the minimal model because it uses only very basic life history and demographic information) developed by NUNNEY and ELAM (1994) to evaluate  $N_c/N$  in red drum. The model requires the following information: the proportion of males in the population ( $r$ ), mean generation time ( $G$ ), mean maturation time ( $M$ ), average life span of males and females ( $A$ ), standardized variance in life span ( $I_A = S$  [survivorship rate from above]), standardized variance in female reproductive success in excess of Poisson ( $I_f$ ), and standardized variance in male reproductive success ( $I_m$ ). Standardized variance in reproductive success is defined as the variance in progeny number divided by mean progeny number squared. We verified that  $A$  and  $I_A$  were very similar for male and female red drum by evaluating catch data separately for each sex and making the simplifying assumption that the values were equal across sexes. Males become sexually mature at about age 4 and females at age 5 (PATTILLO *et al.* 1997). We averaged these values to obtain  $M = 4.5$  years.

$N_c/N$  is most likely reduced by factors that influence variance in reproductive success: Examples include biased sex ratio and mating system (NUNNEY 1993). To determine whether sexes were present in equal numbers, total numbers of reproductive males and females were tallied across 7 years of sampling (1986–1992) and compared using a paired  $t$ -test. Because there are no data available to estimate standardized variances of female and male reproductive success or mating system directly in red drum, we explored the effect of female and male reproductive success as follows. We began by recognizing that standardized variance in female reproductive success has three components (NUNNEY and ELAM 1994): (i) mean number of progeny surviving to reproductive maturity per female ( $b_f$ ), (ii) mean productivity of females under Poisson expectation ( $x = b_f/[1 - \exp(-b_f)]$ ), and (iii) the proportion of females with at least one offspring that survives to maturity ( $p$ ). We set  $b_f = 2$  for a nongrowing population, which resulted in  $x = 2.313$ . Standardized variance in reproductive success was estimated as  $I_f \approx (1 - \alpha_f)/\alpha_f$ , where  $\alpha_f = xp/b_f$  (NUNNEY and ELAM 1994). Because  $x$  and  $b_f$  are fixed in a nongrowing population, we manipulated  $p$  to understand the effects of standardized variance in female reproductive success on  $N_c/N$  under the minimal demographic model.

To evaluate effects of male variance in reproductive success on  $N_c/N$ , it was necessary to hypothesize a mating system for red drum. Based on available ecological data (PATTILLO *et al.*

1997), we hypothesized that the mating system in red drum approximates lottery polygyny. Lottery polygyny can be viewed as a mating system where receptive females randomly encounter males, and upon each encounter, the probability of mating is fixed (NUNNEY 1993). Strictly speaking, lottery polygyny requires that females mate only once (NUNNEY 1993). Red drum females appear to mate more than once (WILSON and NIELAND 1994), but probably do not have nearly as many opportunities for mating with different partners as do males. Lottery polygyny implies that  $I_m = I_f$  if  $r = 0.5$  (NUNNEY 1993, Equation 7). We solved for  $I_f$  and  $I_m$  by substituting empirically derived estimates of  $N_e/N$  obtained from the temporal and coalescent methods into the minimal model.

**Metapopulation structure and  $N_e/N$ :** Under otherwise idealized population assumptions (inbreeding),  $N_e$  in a structured population is a function of inbreeding within each deme ( $F_{IS}$ ) and nonrandom mating among demes ( $F_{ST}$ ), namely,

$$\frac{N_e}{N} = \frac{1}{(1 + F_{IS})(1 - F_{ST})}, \quad (4)$$

where  $N$  is the total number of adults summed across demes (NUNNEY 1999, Equation 15). Note that  $N_e/N$  takes a minimum value of 0.5 when  $F_{IS} = 1$  and  $F_{ST} = 0$  and a maximum value of infinity as  $F_{ST}$  approaches 1. This formula assumes equal productivity among demes.

When local inbreeding, variance in individual reproductive success, and variance in productivity among demes are incorporated into the model, the result is

$$\begin{aligned} \frac{N_e}{N} = 1 / [ & (1 + F_{IS})(1 + F_{ST}) - 2F_{IS}F_{ST} \\ & + I_{kf}[(1 + 3F_{IS})(1 + 3F_{ST}) - 12F_{IS}F_{ST}] \\ & + \frac{1}{2} I_m(1 + I_{ks})(1 + 7F_{IS})(1 - F_{ST}) + I_{ks}(1 + 2N_e F_{ST} + 3F_{IS}) \end{aligned} \quad (5)$$

(NUNNEY 1999, Equation 25):  $I_{kf}$  is variance in female reproductive success attributable to variance among individuals plus variance in productivity among demes,  $I_{ks}$  is variance in productivity among demes, and  $N_e$  is the average adult census size within demes.  $N_e/N$  is lower than unity under all conditions, except when  $F$ -statistics and  $I$  values equal zero (*i.e.*, an idealized population; WHITLOCK and BARTON 1997; NUNNEY 1999).

To examine potential effects of population structure on  $N_e$ , we calculated  $F$ -statistics by using WEIR and COCKERHAM'S (1984) estimator ( $\theta$ ) as implemented in FSTAT (GOUDET 2000), under the assumption that mutation follows an infinite-alleles model. Because a stepwise mutation model may be appropriate for DNA microsatellites, we also estimated  $R_{IS}$  and  $R_{ST}$  (SLATKIN 1995). We substituted  $F$ - and  $R$ -statistics into Equation 5, along with standardized variances  $I_{kf}$  ( $= I_f + I_{ks}$ ),  $I_m$ , and  $I_{ks}$ . For purposes of analysis,  $I_f$ ,  $I_m$ , and  $I_{ks}$  were set to be equal, and  $N_e$  was set to 10,000. As with the minimal demographic model above, we manipulated  $p$  to evaluate the simultaneous effects of standardized variance in female and male reproductive success and variance in productivity among demes. Resulting estimates of  $N_e$  were not strictly correct for red drum because spatial population structure is best described by a modified stepping-stone model rather than a hierarchical island model (GOLD *et al.* 2001). However, these equations were used to provide a rough estimate of the effects of population structure on  $N_e$ .

## RESULTS

Tests for conformation to Hardy-Weinberg equilibrium (HWE) revealed that one of eight microsatellite loci (*Soc 252*) exhibited significant deviations from ex-

pected values in 16 of 28 samples (four year classes at seven geographic sample localities) following sequential Bonferroni correction for simultaneous tests (GOLD and TURNER 2002). We suspect the deviations from HWE result from the presence of one or more null alleles at this locus. To evaluate whether locus *Soc 252* biased estimates of  $N_e$ , we conducted separate analyses with the full data set and with *Soc 252* excluded.

We observed temporal shifts in allele frequencies at all eight microsatellite loci across the four year classes (cohorts) of red drum. Standardized variances in shifts of allele frequencies across cohorts are given in Table 1. On the basis of demographic data and two survivorship ( $S$ ) values, we calculated the following values for  $C$  and  $G$ : When  $S = 0.42$ ,  $C = 17.28$  and  $G = 5.92$ , and when  $S = 0.84$ ,  $C = 58.49$  and  $G = 12.16$ . The lower  $S$  value resulted in proportionally lower values of  $N_e$  when substituted into Equation 3 (data not shown) and we did not consider the low survivorship scenario further. The high  $S$  value resulted in higher age-specific survival rates and, in turn, greater contribution of gametes from older age classes to progeny (Figure 2) and higher  $N_e$ . Variance effective size for the entire population of red drum in the northern Gulf was  $N_e = 2365$  for the moment-based estimator, with lower and upper 95% confidence intervals of 833 and infinity, respectively. Variance effective size for the entire population estimated via the pseudo-ML approach (WANG 2001), and corrected for overlapping generations, was  $N_e = 3516$ , with 95% confidence intervals of 1785 and 18,148, respectively (temporal-method analyses with locus *Soc 252* excluded yielded nearly identical, but less precise values of  $N_e$ ). Dividing observed values of  $N_e$  by the current estimate ( $3.4 \times 10^6$ ; see MATERIALS AND METHODS) of adult census size generated  $N_e/N$  ratios of 0.0007 (moment-based) and 0.001 (pseudo-ML), respectively. Observed  $N_e$  values were at least three orders of magnitude lower than current adult census size.

$\Theta$ , estimated via the coalescent approach employed in MIGRATE, was tabulated by locus and deme (Table 2). Integration over likelihood surfaces obtained separately for each locus and deme permitted estimation of multilocus  $\Theta$  for each deme. These values ranged from  $\Theta = 0.0074$  in Tampa Bay, Florida to  $\Theta = 0.0207$  in Laguna Madre, Texas. Multilocus  $\Theta$  was translated into  $N_e$  for each deme (using  $N_e = \Theta/4\mu$ , where  $\mu = 1 \times 10^{-4}$ ) and then summed across demes to result in a long-term, inbreeding  $N_e$  of 1853 (Table 2) for the entire red drum population in the northern Gulf. The per gene mutation rate was scaled across loci by using empirical minimum and maximum values ( $\mu = 1 \times 10^{-5}$  and  $\mu = 1 \times 10^{-3}$ , respectively) to derive a minimum value  $N_e = 317$  and a maximum  $N_e = 7226$ .

Analysis of catch data revealed that sex ratio did not differ significantly from unity ( $P = 0.54$ , two-tailed paired  $t$ -test); consequently,  $r = 0.5$  was used in the minimal demographic model. Values of other fixed variables used in the model were average life span ( $A$ ) of

**TABLE 1**  
Standardized variance of shifts in allele frequency ( $F$ ) estimated by the temporal method

Locus	Pairwise comparison					
	86 vs. 87	86 vs. 88	86 vs. 89	87 vs. 88	87 vs. 89	88 vs. 89
<i>Soc11</i>	0.004	0.007	0.003	0.006	0.002	0.003
<i>Soc19</i>	0.005	0.006	0.007	0.003	0.005	0.003
<i>Soc35</i>	0.005	0.004	0.004	0.007	0.003	0.005
<i>Soc60</i>	0.006	0.009	0.001	0.015	0.007	0.009
<i>Soc156</i>	0.006	0.005	0.006	0.004	0.004	0.005
<i>Soc204</i>	0.008	0.008	0.007	0.006	0.002	0.003
<i>Soc243</i>	0.002	0.004	0.008	0.005	0.006	0.003
<i>Soc252</i>	0.006	0.006	0.009	0.005	0.005	0.006
Mean $F$	0.005	0.006	0.006	0.006	0.004	0.004
SD $F$	0.002	0.002	0.003	0.004	0.002	0.002

$F$  is reported for all possible pairwise comparisons of allele frequencies at eight microsatellite loci across four year classes (cohorts) of red drum (1986–1989), pooled across seven localities sampled in the northern Gulf of Mexico. Pairwise values were averaged to estimate variance effective size for the entire red drum population. Estimates of  $F$  appear consistent across year classes and loci.

10.75 years, standardized variance of life span ( $I_A$ ) of 0.84, and generation time ( $G$ ) of 12.16 years. Under a lottery polygyny mating system, standardized variances of female and male reproductive success were determined by  $p$ , the proportion of females each year that give birth to at least one offspring that survives to maturity. When values of  $p$  were decreased,  $N_e/N$  also decreased (Figure 3). The value of  $p$  required to solve for  $N_e/N = 0.001$  (empirically derived from the pseudo-ML-based temporal method) was roughly 0.00007 (Figure 3), which is equivalent to 1 in 14,300 females successfully breeding per year.

Hierarchical analysis indicated that genetic variance attributable to local inbreeding ( $F_{IS} = 0.044$ ) was larger

than variance attributable to differences among demes ( $F_{ST} = 0.003$ ). Similar values were obtained by using a method (SLATKIN 1995) that employs a stepwise mutation model ( $R_{IS} = 0.098$ ,  $R_{ST} = 0.004$ ). Substitution of  $F$ - and  $R$ -statistics into Equation 4 yielded  $N_e/N = 0.96$  ( $F$ -statistics), and  $N_e/N = 0.91$  ( $R$ -statistics). Substitution of  $F$ -statistics into Equation 5, with  $I_f = I_m = I_b$  and their values dependent on values of  $p$ , showed a marked decrease of  $N_e/N$  compared to the minimal demographic model for the same values of  $p$  (Figure 3).  $R$ -statistics yielded very similar results (not shown). A value of  $p = 0.058$ , which is roughly equivalent to 1 in 17 females successfully breeding per year, was sufficient to generate  $N_e/N = 0.001$  in the hierarchical metapopulation model

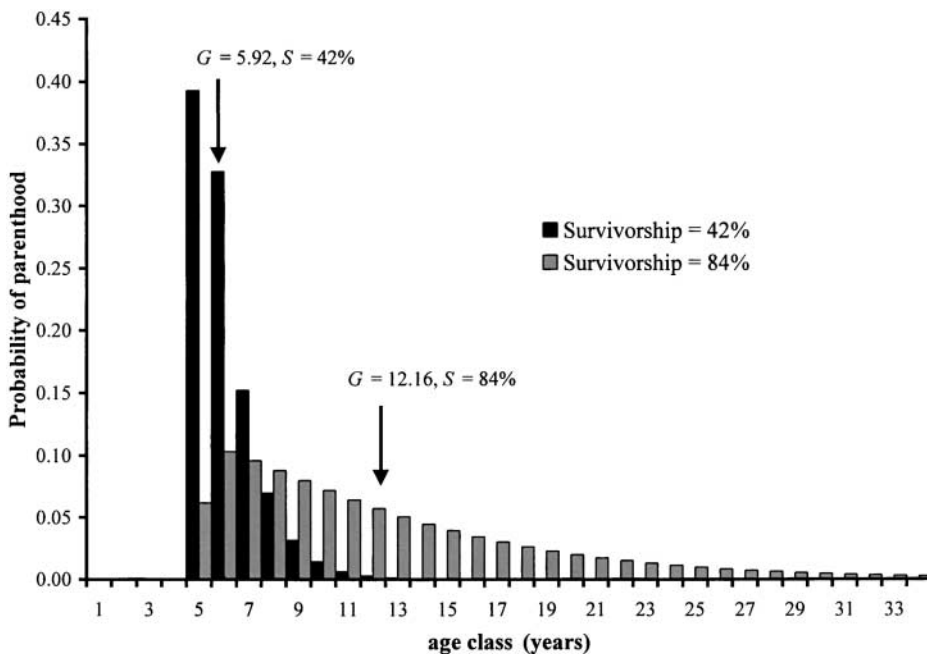


FIGURE 2.—The probability that members of age class  $i$  are the parents of individuals in a given cohort. This was generated from life table information described in the text. Two values of survivorship ( $S$ ) are depicted, and note that increasing survivorship increases the probability of parenthood for members of older age classes.

**TABLE 2**  
**Coalescent method estimates of  $\Theta$  and  $N_e$  for each sample locality (year classes pooled)**

Locality	Locus								$N_e$
	<i>Soc11</i> ( $\Theta$ )	<i>Soc19</i> ( $\Theta$ )	<i>Soc35</i> ( $\Theta$ )	<i>Soc60</i> ( $\Theta$ )	<i>Soc156</i> ( $\Theta$ )	<i>Soc204</i> ( $\Theta$ )	<i>Soc243</i> ( $\Theta$ )	<i>Soc252</i> ( $\Theta$ )	
Tampa Bay, FL	0.079	0.084	0.167	0.143	0.067	0.044	0.037	0.095	185
Apalachicola, FL	0.131	0.121	0.140	0.062	0.099	0.032	0.153	0.147	184
Biloxi Bay, MS	0.057	0.163	0.166	0.084	0.302	0.158	0.188	0.155	296
Grand Isle, LA	0.046	0.122	0.116	0.093	0.039	0.109	0.052	0.134	207
West Bay, TX	0.202	0.191	0.176	0.077	0.134	0.202	0.041	0.121	243
Pass Cavallo, TX	0.051	0.126	0.082	0.099	0.119	0.034	0.102	0.141	221
Laguna Madre, TX	0.118	0.312	0.266	0.079	0.206	0.225	0.124	0.315	517
Northern Gulf of Mexico									1853

$N_e = \Theta/4\mu$  is calculated using a mutation rate ( $\mu$ ) of  $1 \times 10^{-4}$  obtained by averaging across scaled estimates of  $\mu$  for each microsatellite.  $\Theta$  for each population was obtained by summing over sampled genealogies and likelihood surfaces. Estimates of  $\Theta$  obtained by integrating over all microsatellites are not shown, only the translation of this value into  $N_e$ .

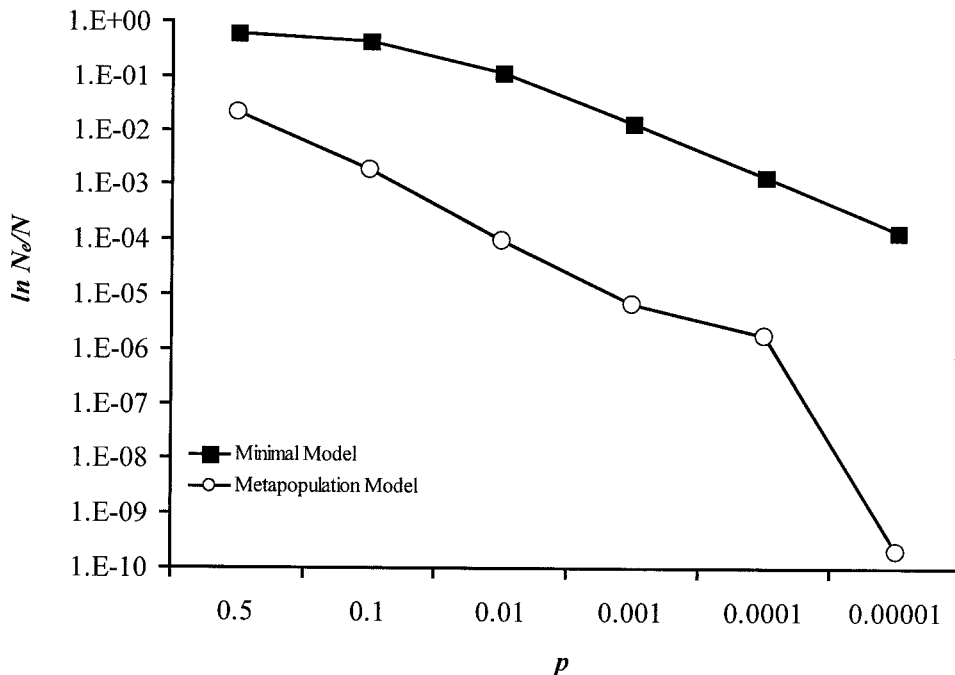
depicted in Equation 5 (Figure 3). This value is three orders of magnitude greater than  $p$  required to solve for  $N_e/N$  in the minimal demographic model.

**DISCUSSION**

Regardless of analytical approach used, empirical estimates of  $N_e$  for red drum in the northern Gulf of Mexico are at least three orders of magnitude less than current estimates of adult census size ( $N$ ); *i.e.*,  $N_e/N \leq 0.001$ . This result is surprising, as red drum is a species characterized by a long reproductive life span and overlapping generations. Minimally, this suite of life history traits is expected to increase  $N_e/N$  by limiting variance in life-

time reproductive success among individuals (HILL 1979; JORDE and RYMAN 1995; WAITE and PARKER 1996). However, there are at least two factors expected theoretically to lower genetic effective size with respect to adult census size in species like red drum. These include (i) fluctuating adult population numbers and (ii) factors that influence variance in reproductive success. The latter include biased sex ratio, a mating system that produces high variance in male and/or female reproductive success, and variance of productivity among habitats (summarized in Table 3).

In an otherwise idealized population, fluctuation of adult population number over time is expected to lower  $N_e$  relative to estimates of adult census size (VUCETICH



**FIGURE 3.**—The (log) ratio  $N_e/N$ , as estimated by the minimal demographic model of NUNNEY and ELAM (1994) and by the hierarchical metapopulation model of NUNNEY (1999), plotted by the proportion of red drum females that leave at least one offspring that reaches sexual maturity ( $p$ ). A value of  $p = 0.00007$  (and, thus, very large values of individual variance in reproductive success) is required to generate  $N_e/N$  values observed in red drum in the minimal demographic model. This model does not account for spatial population structure and variability of productivity among demes. For the metapopulation model, a value of  $p = 0.058$  is sufficient to generate observed  $N_e/N$  values in red drum owing to interactions with variance in productivity among spatially structured demes.

TABLE 3

An evaluation of factors that could lower the genetic effective size to adult census size ratio ( $N_e/N$ ) in red drum (*Sciaenops ocellatus*)

Deviation from idealized population	Predicted effect on $N_e/N$ (with respect to idealized population)	Observation or statistical test	Hypothesized effect on observed $N_e/N$ ?
Fluctuating population size	Decrease	$N$ (1987) $\approx N$ (1998); temporal method $N_e \approx$ coalescent method $N_e$	Small
Biased sex ratio	Decrease	Sex ratio does not deviate from 1:1 ( $P = 0.52$ ; $t$ -test)	Small
Nondiscrete ( <i>i.e.</i> , overlapping) generations	Increase	Correction increases $N_e$ by factor of 4.81	Small
Population structure (migration-drift equilibrium with local inbreeding)	Decrease	$N_e/N = 0.96$ when $F$ -statistics were substituted into equilibrium models	Small
Metapopulation structure (with variance in productivity among demes)	Decrease	See text	Large
Variance in reproductive success among individuals	Decrease	$P = 0.00007$ required to solve for observed $N_e/N$ ; unrealistically small	Small
Variance in productivity among demes	Decrease	$P = 0.058$ required to solve for observed $N_e/N$ , provided that variance in productivity is equal to variance in individual reproductive success	Large

*et al.* 1997). However, such fluctuations affect variance and inbreeding effective sizes in different ways. Variance effective-size estimates based on the temporal method are influenced by population bottlenecks only if one (or both) of two temporally spaced samples used to estimate variance in allele frequency shifts is (are) obtained during bottleneck events (RICHARDS and LEBERG 1996; LUIKART *et al.* 1999). This is because the estimate of  $N_e$  depends solely on shifts in allele frequencies over the sampling period (HUSBAND and BARRETT 1992); *i.e.*, temporal-method estimates are insensitive to bottlenecks that occurred prior or subsequent to sampling. Conversely, inbreeding effective size, as estimated by a coalescent approach, is influenced strongly by historical bottleneck events. This is because genetic variants (alleles) are removed from a small population by genetic drift much faster than new genetic variants arise via mutation. In addition, because a coalescent approach estimates long-term inbreeding effective size, the timescale over which population fluctuation is expected to affect estimates of  $N_e$  is equivalent to the time necessary to reestablish the equilibrium between genetic drift and mutation (AVISE 2000).

Census data (NICHOLS 1988; MITCHELL and HENWOOD 1999), taken over the time period covered in this study, indicate that the number of adult red drum in the northern Gulf has remained essentially unchanged over at least one red drum generation. We interpret this to mean that fluctuations in adult population number may not be solely responsible for the low value(s) of  $N_e/N$  derived from the temporal method. It should be noted

that age structure was not constant over this period (WILSON and NIELAND 1994), suggesting that variability in recruitment success across year classes may influence our estimates of  $N_e$  (we assumed stable age structure). To our knowledge, there are currently no models available to simultaneously evaluate effects of variability in recruitment and overlapping generations on  $N_e$ , despite the fact that recruitment variability is ubiquitous in marine fishes (FOGARTY *et al.* 1991).

Comparison of short-term variance  $N_e$  (via the temporal method) and long-term inbreeding  $N_e$  (via coalescent analysis) based on microsatellites yielded nearly identical estimates of  $N_e$ . We interpret this to suggest that neither contemporary nor historical fluctuations in adult population size alone are sufficient to account for the low ratio of  $N_e/N$  observed. Moreover, near identity of temporal-method and coalescent-based estimates of  $N_e$  suggests that factors that reduce  $N_e/N$  in contemporary red drum operated similarly in the past.

If historical and recent fluctuations in adult census size are not sufficient to explain the observed low value of  $N_e/N$  in red drum, then variance in reproductive success in either or both sexes and/or variance in productivity among subpopulations must be important factors. Sex ratios across 7 years of red drum catch data (WILSON and NIELAND 1994) did not differ significantly from unity, indicating that sex-ratio differences likely do not contribute to the low  $N_e/N$ . We then used a minimal demographic model (NUNNEY and ELAM 1994) to estimate the proportion of successful females necessary to account for the low values of  $N_e/N$  observed. If mating



system in red drum approximates lottery polygyny (*i.e.*, female and male variance in reproductive success are equal), then the proportion of females that successfully produce an offspring that reaches sexual maturity ( $p$ ) equals the proportion of successful matings per year. For this model to adequately explain empirically derived  $N_e/N$  values, only 1 mating pair out of every 14,300 matings are successful (*i.e.*, produce offspring that survive to maturity). This variance in reproductive success seems unrealistically large (see NUNNEY 1996), but if true, would imply that across the northern Gulf only 240 or so mating pairs per year (out of 1.7 million potential mating pairs) produce offspring that survive to maturity. Such a large variance in individual reproductive success is possible in red drum if one considers the enormous reproductive capacity per female (PATTILLO *et al.* 1997). Altogether, these results indicate that a stable, nongrowing, adult population of several million individuals could be sustained if  $\sim 0.7\%$  of the offspring potentially produced from each of 240 matings per year grew to maturity and reproduced successfully.

The minimal demographic model of NUNNEY and ELAM (1994) does not account for hierarchical population structure observed in red drum, and thus, probably does not provide a realistic picture of the forces that drive  $N_e/N$  to low values in this species. Temporally stable population genetic structure exists in the northern Gulf, and we have hypothesized that this structure occurs, at least in part, because the species depends critically on patchily distributed estuarine habitats for successful recruitment (GOLD *et al.* 2001). It is clear from studies of red drum and other estuarine-dependent species that estuaries vary greatly in their productivity and suitability for recruitment of young-of-the-year fishes. Environmental factors like temperature, annual precipitation (and associated freshwater inputs), and the severity of toxic algae blooms vary spatially and temporally among bays and estuaries in the Gulf (MOTE 1996; PATTILLO *et al.* 1997). Local environmental conditions have been shown to very strongly affect recruitment in estuarine-dependent species, with adverse conditions leading to recruitment failure (PATTILLO *et al.* 1997; WITTING *et al.* 1999; LANKFORD and TARGETT 2001).

NUNNEY (1999) employed a hierarchical metapopulation model to consider the effects of population structure, individual variance in reproductive success, variance in productivity among demes, and the interactions of these factors on the ratio  $N_e/N$ . This model likely provides a more realistic framework for considering factors that drive  $N_e/N$  to low values. Under otherwise idealized conditions, hierarchical population structure alone appeared to be insufficient to explain low values of  $N_e/N$ , as substitution of  $F$ -statistics into Equation 4 resulted in  $N_e/N$  values near one. However, examination of Equation 5, which provides for partitioning variance among sources attributable to reproductive success differences among females and males and productivity differences

among demes, revealed that if all of these sources of variance are equal in magnitude, then 1 out of 17 matings per year must produce offspring that survive to maturity to account for  $N_e/N = 0.001$ . We then sought to understand the importance of local inbreeding, spatial structure, and each source of variance individually by setting values of  $F_{IS}$ ,  $F_{ST}$ ,  $I_{kt}$ ,  $I_m$ , and  $I_{ks}$  to zero sequentially (without changing other values) and each time evaluating the effect on  $N_e/N$ . We expected that when important variables (and their interactions) were omitted by setting them to zero, then the result of Equation 5 would be raised substantially  $> N_e/N = 0.001$ . Setting  $F_{IS}$ ,  $I_{kt}$ , or  $I_m$  to zero raised  $N_e/N$  values only slightly  $> 0.001$ . However, setting  $F_{ST}$  to zero increased  $N_e/N$  by an order of magnitude, and setting  $I_{ks}$  to zero increased  $N_e/N$  by two orders of magnitude. This analysis points to an interaction of variance in productivity and genetic divergence across demes as a critical factor relative to explaining low  $N_e/N$  observed in red drum in the northern Gulf of Mexico.

The concept that high variance in reproductive success may reduce genetic effective size in species like red drum is not new. Motivated by the empirical finding that  $N_e/N$  was five orders of magnitude lower than expected in a population of Pacific oysters, HEDGECOCK (1994) proposed a "sweepstakes" process whereby larvae from females that have enormous reproductive potential experience high mortality as they disperse into highly heterogeneous environments. Hedgecock envisioned that very large variances in reproductive success (far in excess of Poisson expectation) could result from this process and ultimately yield very low  $N_e/N$ . NUNNEY (1996) criticized these findings, arguing on theoretical grounds that such high variances in reproductive success are unlikely and that the low value of  $N_e/N$  observed for Pacific oysters probably resulted from allele frequency shifts facilitated by migration of new individuals into the study area. Immigration of new alleles is an unlikely explanation for observed allele frequency shifts in red drum because we have sampled the species over most of its range in the northern Gulf. Rather, we hypothesize that variance in productivity among demes (WHITLOCK and BARTON 1997; NUNNEY 1999) explains the observed low ratio of  $N_e/N$ .

Unless reliable estimates of variance in individual reproductive success and/or variation in productivity among demes are available, the low values of  $N_e/N$  empirically derived for red drum in the northern Gulf suggest an important caution for sole reliance on demographic models to estimate  $N_e$  in the absence of genetic data (NUNNEY and ELAM 1994) or with only limited genetic data (BASSET *et al.* 2001). Our study indicates, albeit indirectly, that variance in productivity among critical habitats necessary for successful recruitment is probably very large and plays a significant role for determining genetic diversity in red drum. Thus, reliable estimation of variance in productivity among bays and

estuaries will be an important step in predicting  $N_e/N$  for fish and other (e.g., invertebrate) species that like red drum are dependent on bay and estuarine habitats. Linking ecology, demography, and genetic diversity also will likely be important for sustaining critical marine fish resources. In the United States, for example, an estimated 50% of the total marine fish harvest is estuarine dependent at one or more critical life history stages (HOUE and RUTHERFORD 1993). Our work suggests that genetic resources in commercially exploited estuarine-dependent fishes may be much lower than previously appreciated and that any action that increases variance in productivity among bays and estuaries may result in further depletion of this genetic diversity.

Nearly identical values of short-term variance  $N_e$  (from the temporal method) and long-term  $N_e$  (from coalescent methods) suggest that the variance in productivity among demes that drives  $N_e/N$  to low values in red drum in the northern Gulf has acted similarly in the past. This observation has important implications for maintenance of genetic diversity in marine species, including red drum, that depend critically on patchily distributed resources. Habitat alteration or disturbance within a bay or estuary that affected recruitment success would be expected to increase variance in productivity among demes and consequently to lower  $N_e/N$ . Traditionally, causal mechanisms for decreasing genetic diversity in economically important marine species like red drum have been tied to overfishing of adults (TREXLER and TRAVIS 2000). Our findings suggest that low  $N_e/N$  values also may derive from events occurring during early stages of recruitment and the variance in critical resources at this life stage.

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