What Kind of Genetic Information is Best for Estimating Genetic Effective Population Size? A Case Study of Three Classes of Molecular Markers Surveyed in Red Drum (*Sciaenops ocellatus*) from the Gulf of Mexico

THOMAS F. TURNER and JOHN R. GOLD
Center for Biosystematics and Biodiversity
Texas A&M University
College Station, Texas 77843-2258 USA

ABSTRACT

Genetic information from managed fish populations is often used to estimate genetic effective population size, N_e. Managers must weigh the cost of genetic study as determined in part by sampling effort, against potential benefits that are determined by the hypotheses that the data can address adequately (i.e. statistical power). This paper is a case study that examines statistical power to estimate N_e in red drum (*Sciaenops ocellatus*) through the use of genetic markers. Extensive genetic information available for this species permitted comparison of three commonly used classes of genetic markers: (i) allozymes; (ii) mitochondrial (mt) DNA; and (iii) microsatellite DNA loci. Statistical power analysis indicated that all three classes of genetic markers can estimate small genetic effective population sizes ($N_e < 100$) with high accuracy and statistical confidence, given a large number of individuals sampled. As N_e increases above 500, statistical power and accuracy differ among markers: mtDNA performed best, followed by microsatellites, and then allozymes. Power is higher for mtDNA because the effective size of the mtDNA locus is one-quarter the effective size of diploid, nuclear loci (i.e., microsatellites and allozymes). For nuclear markers, increasing number of independent DNA microsatellite loci increases statistical power more effectively than increasing the number of allozyme loci. An indirect estimate of N_e from mtDNA restriction site data indicates that effective population size of red drum is sufficiently large to preclude critical losses of genetic diversity to drift despite recent declines in abundance.

KEY WORDS: Variance effective population size, microsatellites, red drum

INTRODUCTION

Genetic data are used extensively in fisheries management to monitor levels of genetic diversity in natural populations (stocks) and for identification of genetically distinct stocks of commercially and recreationally harvested fishes (Ryman and Utter 1988). Attention also has focused on estimation of genetic effective population size (N_e), because this parameter determines the rate at

which genetic diversity is lost to genetic drift. Low levels of genetic diversity in normally outbreeding species may reduce fitness by increasing susceptibility to disease or environmental perturbation. In applicable cases, N_e predicts the relative impact of hatchery supplementation on genetic diversity in wild stocks (Ryman and Laikre, 1991). Lastly, declines in abundance from over harvesting are expected to reduce values of N_e from historical levels: N_e thus can provide important baseline data for future monitoring of effective population size.

Despite its importance, estimation of N_e for many marine fish species is difficult. Precision of the estimate depends on the sampling effort relative to the size of the population being studied, i.e. estimates for large, geographically widespread species require large sample sizes. If genetic methods are used to determine N_e, it is possible to increase statistical power and precision by choosing genetic markers with high levels of variability. However, it is difficult to know *a priori* the strengths and limitations of different classes of genetic markers, and the choice of markers often is limited by the funds available for study.

This study describes statistical power and precision of estimates of N_e derived from three commonly used classes of genetic markers: (i) allozymes, (ii) mitochondrial DNA restriction site polymorphisms (mtDNA), and (iii) microsatellite DNA loci. The study is based on genetic data gathered for red drum (*Sciaenops ocellatus*), an estuarine-dependent species that supports an important fishery in the Gulf of Mexico. Red drum provide an excellent case study because patterns of genetic variability, life history, and estimated census sizes are similar to many other commercially important fish species in the Gulf of Mexico (Ramsey and Wakeman, 1987; Gold *et al.*, 1993; Patillo *et al.*, 1997). We use comparisons among marker classes to make general recommendations for estimating N_e. Finally, we provide an estimate of N_e for red drum based on mtDNA restriction site data and discuss its interpretation in light of results of statistical power analysis.

MATERIALS AND METHODS

Three different classes of genetic markers were examined in this study. Allozymes are protein products of nuclear structural genes and usually are inherited in a codominant, Mendelian fashion (Murphy *et al.*, 1990). Allozymes have been used extensively for several decades to characterize genetic variability in a wide variety of organisms (Avise, 1994). Patterns of genetic variability of allozymes in red drum from the Gulf of Mexico and Atlantic Ocean were assessed from a study by Bohlmeyer and Gold (1991).

The second class of markers examined was restriction site polymorphisms of the entire mitochondrial (mt) DNA molecule. MtDNA differs from allozymes in

that it is maternally inherited as a single haploid locus. Because of maternal haploid inheritance, effective population size of mtDNA is one quarter the effective size of a nuclear diploid locus. MtDNA is usually more variable than allozymes because mutation rates of mtDNA are generally higher than nuclear structural genes (Brown, 1983). Patterns of restriction site variation in red drum sampled from the 1986 and 1987 year classes in the Gulf of Mexico were taken from published studies (Gold and Richardson, 1991; Gold *et al.*, 1993). Additional individuals from the 1988 and 1989 year classes, surveyed for the same restriction enzymes, also were examined.

The third class of markers examined were microsatellites, short simple sequence repeats contained in nuclear DNA of most eukaryotes (Brooker *et al.*, 1994). Like allozymes, microsatellites are encoded in the nucleus and inherited in a codominant, Mendelian fashion. However, microsatellites evolve much faster than allozymes, and as a result, proportionally more microsatellite loci are polymorphic, have higher heterozygosities, and possess more alleles per locus than do allozymes. Ten polymorphic microsatellite loci where chosen from a total of thirty developed for red drum (Turner *et al.*, 1998). Each locus was screened for variation in red drum collected from Apalachicola Bay, in the northern Gulf of Mexico, and Mosquito Lagoon and Charleston Harbor in the southern Atlantic Ocean.

The number of polymorphic loci and average number of alleles (haplotypes) per locus were calculated for each class of markers. For mtDNA restriction site data, we calculated the average number of alleles (haplotypes) present in comparisons between year classes. The rationale for this is that some haplotypes occurred in only a single individual in a single year class. N_e is estimated from pairwise comparisons and use the total number of alleles observed. Including all haplotypes would have inflated the actual number of alleles available for estimating N_e. The number of loci and alleles for each class of markers were used to compare their performance in estimating N_e.

Of the several methods available to estimate N_e (Nunney and Elam, 1994), we chose the temporal method (Pollak, 1983) because: (i) it is the only method applicable to all three classes of genetic markers, and (ii) its statistical properties are well known (Waples, 1989). The temporal method estimates the variance in the shift of allele frequencies over time, and thus requires two consecutive samples of the same population. When corrected for sampling variance, the temporal variance in allele frequency shifts is inversely related to N_e in an idealized population (Pollak, 1983).

The sampling variance is an error term that results from the sampling of the population under study. It must be subtracted from the total variance to estimate N_e. Sampling variance also must be subtracted from upper and lower bound

confidence intervals of the total variance in order to estimate statistical confidence intervals around N_e. If sampling variance is larger than the lower-bound estimate of total variance, the result is negative. This means that the sampling design was insufficient to discriminate the population under study from an infinitely large population, i.e., has low statistical power. For our study, statistical power was defined as the expected probability that the experimental sample size and genetic marker will yield a finite upper bound estimate of N_e. Precision is defined as the width of the 95% confidence interval around N_e after correction for sampling error.

Our study examined statistical power to test the null hypothesis that $N_e = \infty$, and 95% confidence intervals around estimates of N_e, for each of three different classes of genetic markers. The total temporal variance (drift variance plus sampling variance) in the shift of allele frequencies over one generation was determined for populations of sizes N_e = 10, 100, 500, 1,000, and 10,000 for each class of markers. Sampling variance was estimated by using a sample size of 300 individuals per generation (chosen to reflect actual sample sizes obtained for red drum). Upper and lower bound confidence intervals for total temporal variance were estimated by using the number of loci and alleles calculated for each marker class and by employing the formula in Sokal and Rohlf (1981, pg. 159). Confidence intervals calculated in this way assume that temporal variance is distributed approximately as a chi-square, an assumption that was verified in Monte Carlo simulations of up to 20 loci with two and three alleles, respectively (Waples 1989).

Finally, we provide an estimate of N_e using the temporal method for red drum collected in the Gulf of Mexico and surveyed for mtDNA restriction site variation. N_{ef}, the effective number of females, was obtained from pairwise comparisons across the 1986-1989 year classes then averaged and multiplied by four, the average time to maturity for red drum females (Waples, 1990). This value was then multiplied by 2 for N_e (males and females) assuming a sex ratio of one.

RESULTS AND DISCUSSION

Three classes of markers were characterized in red drum. Each class of markers had different characteristics: allozymes exhibited a large number of loci but few alleles; mtDNA represents only one locus but with many alleles; and microsatellites have a large number of loci and moderate numbers of alleles (Table 1). Patterns of polymorphism in each marker class were consistent with patterns observed in other marine teleost fishes (Bohlmeyer and Gold, 1991; Gold *et al.*, 1993, unpublished data from our laboratory).

Table 1. Genetic data for three distinct classes of genetic markers, sampled from red drum (*Sciaenops ocellatus*). Data sources are listed below. For purposes of comparison, the number of individuals sampled per year class was held constant across marker types (S = 300). The number of individuals sampled reflects actual sample sizes for mtDNA restriction site polymorphism study on red drum (Gold *et al.*, 1993).

Genetic Marker	Polymorphic loci	Mean Number of alleles per locus	Number of individuals per year class
allozymes [1]	9	4	300
mtDNA[2]	1	100	300
microsatellites[3]	10	15	300

[1]Bohlmeyer and Gold 1991
[2]Gold and Richardson 1991, Gold *et al.* 1993
[3]Turner *et al.*, 1998.

Our results show that when number of individuals sampled are held constant, mtDNA provides the highest statistical power and precision over the broadest range of N_e: under the same conditions, microsatellites performed slightly less well than mtDNA but much better than allozymes (Figures 1 and 2). All three classes of markers performed nearly equally well at $N_e < 100$ in terms of statistical power (Fig. 1) and precision (Figure 2), but the gap between them steadily increased as the size of the (log) effective population increased. At $N_e = 10000$ all three marker classes performed poorly (statistical power ≈ 0.5), suggesting that limits of resolution of all classes of markers had been reached (Fig. 1). Simulation study of statistical power for highly polymorphic systems (i.e., 10 loci with 15 alleles each) produced nearly identical values to those estimated by chi-square (Turner and Gold 1998), suggesting that chi-square provides a good approximation for statistical power even for highly polymorphic loci.

MtDNA performs better than nuclear markers because the effective population size is one-quarter the effective size of a biparentally inherited, diploid (nuclear) locus. Among nuclear loci, microsatellites provided more statistical power over a broader range of N_e than allozymes. For nuclear genetic markers power increases as a function of the number of independent alleles available to estimate N_e: microsatellites possessed 140 independent alleles, and had much higher power than allozymes, with 27 independent alleles.

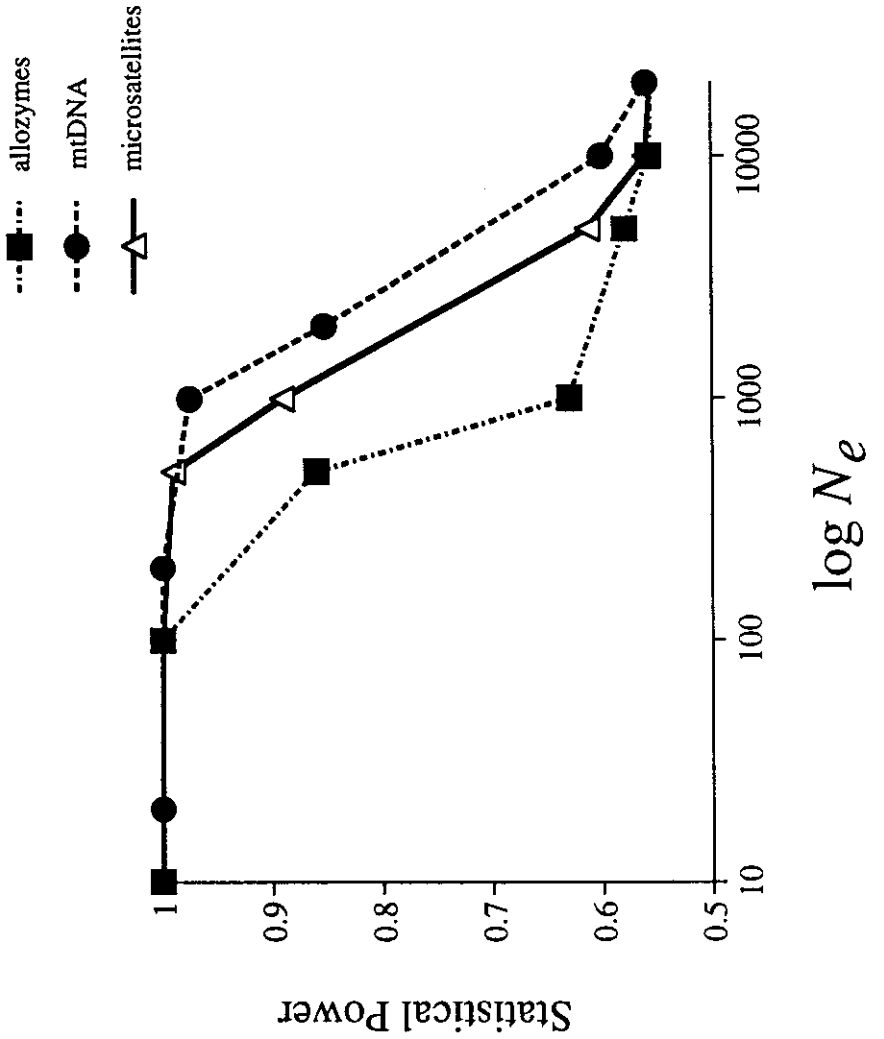

Figure 1. Comparison of statistical power among three different classes of genetic markers. Statistical power (y-axis) is defined as the probability that a given marker class will yield a finite upper bound estimate of N_e. Note that as (log) effective size of the population we wish to detect increases (x-axis), statistical power decreases. Number of individuals sampled per generation (S) was 300, which was held constant.

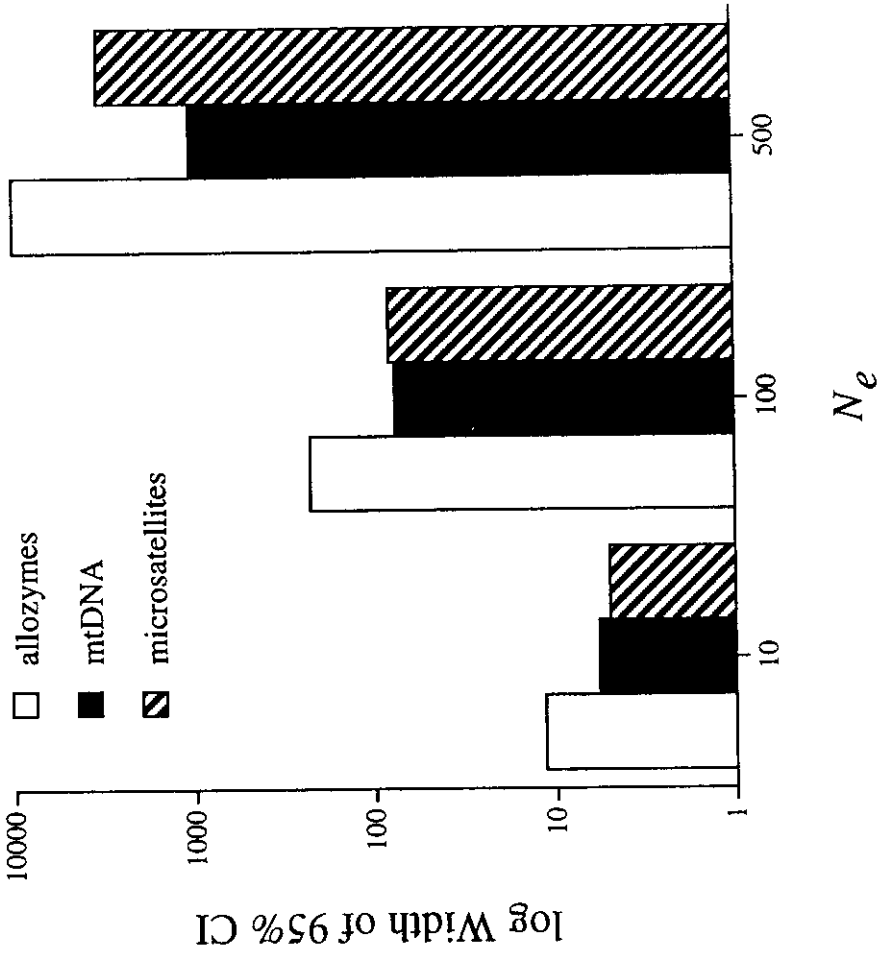

Figure 2. Precision of N_e estimates for three different classes of genetic markers at varying N_e. Precision is defined as the width of the 95% CI (y-axis). Smaller widths reflect higher accuracy. For N_e = 500, the upper bound CI was infinitely large for allozymes. Number of individuals sampled per generation (S) was 300, which was held constant

Genetic effective population size is a critical parameter for effective management, especially for species like red drum. There are at least threes reasons why a robust estimate of N_e is important for this species: (i) to monitor and predict future levels of genetic diversity; (ii) to estimate the relative impact of hatchery supplementation on genetic diversity in wild stocks (Tringali and Bert, in press); and (iii) to determine whether recent declines in abundance have lowered current N_e below historical levels. For red drum and other marine fishes, the problem with estimating N_e is that sampling effort is small relative to the size of the population being studied. Relatively small samples increase sampling variance and decrease the statistical power to estimate N_e. If genetic methods are used, our results show that it is possible, by choosing appropriate markers, to increase statistical power when number of individuals sampled is held constant.

Despite lowered statistical power at large N_e, mtDNA restriction site polymorphism data provided a reasonable estimate of effective population size for red drum in the Gulf of Mexico, viz., N_e = 33,800. As expected from power analysis, the upper bound confidence interval is infinity, and the lower bound is 3880. Thus far, our study has focused on the upper bound CI and the power to distinguish a large population from an infinitely large one. However, the lower bound is equally important. To illustrate this point we compared our lower bound estimates of N_e to benchmark N_e values ostensibly important from a management perspective. Our lower bound estimate exceeds two "critical values" for loss of genetic diversity : (1) $N_e \approx$ 100, the value at which drift removes significant amounts of genetic diversity each generation (Frankel and Soulé 1981); and (2) $N_e \approx$ 500, the value at which high hatchery supplementation from a small brood stock is likely to lower levels of genetic diversity (Ryman and Laikre 1991). Both of these issues are germane to management of red drum.

As in any study, it is important to identify the goals of the research effort in advance and to design a sampling strategy to meet those goals. Our results indicate that for small populations, any of the three marker classes we examined will accurately and confidently estimate N_e. The researcher must determine the level of resolution necessary for the question being addressed. For example, to determine whether a population of red drum is larger than N_e = 100 at α = 0.05 would require roughly 60 individuals per generation if microsatellites were used. However, to estimate a finite upper bound CI (at α = 0.05) of a red drum population of size N_e = 10000 would require nearly infinitely large sample sizes per generation.

Our results suggest that the optimal genetic markers to employ for estimating N_e is mtDNA. However, our results further suggest that all genetic

markers will fail to confidently identify extremely large populations, given typical levels of polymorphism and numbers of individuals sampled. It is thus crucial to determine acceptable lower limits for effective population size and design studies to test whether the target species exceeds those limits.

ACKNOWLEDGEMENTS

We thank J. Bielawski, E. Heist, and L. Richardson for helpful comments on the manuscript. This study is number XXIII in the series "Genetics Studies in Marine Fishes" and is contribution number 71 from the Center for Biosystematics and Biodiversity at Texas A&M University. This project is funded by grants from the Texas Agricultural Experiment Station (Projects H-6703 and REP 3-038) and the Advanced Research Program (ARP) of the State of Texas (Project 999902-103).

LITERATURE CITED

Avise, J.C. 1994. *Molecular Markers, Natural History and Evolution.* Chapman and Hall. New York.

Bohlmeyer, D.A., and J.R. Gold. 1991. Genetic studies in marine fishes II. A protein electrophoretic analysis of population structure in the red drum *Sciaenops ocellatus. Mar. Biol.* **108**:197 - 206.

Brooker, A.L., D. Cook, P. Bentzen, J.M. Wright and R.W. Doyle. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. *Can. J. Fish. Aquat. Sci.* **51**:1959 - 1966.

Brown, W.M. 1983. Evolution of animal mitochondrial DNA. Pages 62-88 in: M. Nei and R.K. Koehn (eds.) *Evolution of Genes and Proteins.* Sinauer, Sunderland, MA.

Frankel, O.H., and M.E. Soulé. 1981. *Conservation and Evolution.* Cambridge University Press, Cambridge, England, 327 p.

Gold, J.R., and L. R. Richardson. 1991. Genetic studies of marine fishes. IV. An analysis of the population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. *Fish. Res.* **12**:213 - 241.

Gold, J.R., L.R. Richardson, C. Furman, and T.L. King. 1993. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. *Mar. Biol.* **116**: 175-185.

Murphy, R. W., J.W. Sites, Jr., D.G. Buth, and C. H. Haufler. 1990. Proteins I: isozyme electrophoresis. Pages 45-126 in: Hillis, D.M., and C. Moritz, (eds.) *Molecular Systematics.* Sinauer, Sunderland, MA.

Nunney, L., and D.R. Elam. 1994. Estimating the effective size of conserved populations. *Conserv. Biol.* **8**:175 - 184.

Patillo, M.E., T.E. Czapla, D.M. Nelson, and M.E. Monaco. 1997.

Distribution and abundance of fishes and invertebrates in Gulf of Mexico estuaries, Volume II: Species life history summaries. ELMR Rep. No. 11 NOAA/NOS Strategic Environmental Assessments Division, Silver Spring, MD. 377 p.

Pollak, E. 1983. A new method for estimating the effective population size from allele frequency changes. *Genetics* **104**:531 - 548.

Ramsey, P.R., and J.M. Wakeman. 1987. Population structure of *Sciaenops ocellatus* and *Cynoscion nebulosus* (Pisces:Scianidae): biochemical variation, genetic subdivision, and dispersal. *Copeia* **1987**:682 - 695.

Ryman, N., and L. Laikre. 1991. Effects of supportative breeding on the genetically effective population size. *Conserv. Biol.* **3**:325 - 329.

Ryman, N, and F. Utter. 1987. *Population Genetics and Fishery Management.* University of Washington Press, Seattle, WA. 420 p.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*. 2nd ed. W.H. Freeman and Co. New York. 859 p.

Tringali, M.D. and T.M. Bert. Risk to genetic effective population size should be an important consideration in fish stock enhancement programs. in press.

Turner, T.F., and J.R. Gold. Statistical power and accuracy of estimates of effective population size differ among three classes of molecular markers in red drum (*Sciaenops ocellatus*). submitted to *Can. J. Fish. Aquat. Sci.*

Turner, T.F., L.R. Richardson and J.R. Gold. Polymorphic microsatellite loci in red drum (*Sciaenops ocellatus*). submitted to *Mol. Ecol.*

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

Waples, R.S. 1990. Conservation genetics of pacific salmon. II. Effective population size and the rate of loss of genetic variability. *J. Hered.* **81**:267 - 276.