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Spatial Homogeneity and Temporal Heterogeneity of Red Drum (*Sciaenops ocellatus*) Microsatellites: Effective Population Sizes and Management Implications

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Abstract: The red drum (*Sciaenops ocellatus*) is one of a number of species that occupy estuarine waters as juveniles and migrate to open ocean waters as adults. This species has experienced dramatic declines in population numbers over the past 2 decades, which has prompted increasing fishery restriction. In addition, hatchery augmentation has been initiated by several states to increase the abundance of juveniles in local areas. In South Carolina hatchery-reared fish have made significant (20%) contributions to the juvenile population on very local scales. As hatchery-reared fish are typically produced by a small number of individuals, the genetic consequences of augmentation programs are of concern. In this article we assess genetic variation at 5 microsatellite loci in *S. ocellatus*. The data indicate little geographic differentiation among samples collected along the Atlantic Coast of the United States, but substantial differences among year classes taken from South Carolina. The gene frequency differences among year classes were used to estimate the effective population size (N_e) of *S. ocellatus* in South Carolina and suggested that N_e was less than 300 from 1990 to 1993 and increased to about 1000 in 1994 and 1995. Whether this increase reflects the effectiveness of management regulations or simply a random fluctuation in *S. ocellatus* populations is not clear. The data suggest that a limited number of individuals produce the bulk of a given year class and support the sweepstakes hypothesis. Given the small N_e and estimates of the contribution of hatchery-reared fish to the wild stock, it is suggested that programs have the potential to increase, rather than decrease; N_e in the wild.

Key words: genetic variation, microsatellites, red drum, effective population size, hatchery augmentation.

INTRODUCTION

The red drum (*Sciaenops ocellatus*) is one of a number of fishes that reside in estuarine waters as juveniles and adopt an oceanic existence as adults. The species is widely distributed along the Atlantic seaboard of the United States and in the Gulf of Mexico from Cape Cod to Laguna Madre, Texas (Pattillo et al., 1997). The adults spawn in deep holes at or near the seaward entrance of estuaries (Holt et al., 1985; Wenner, 2000), and the eggs hatch in about 24 hours. The larvae recruit to nursery areas 5 to 7 days later. The juveniles grow rapidly and reach repro-

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ductive maturity by age 3 or 4, when they leave estuarine waters to join coastal migratory stocks. In the past the species has been important to recreational and commercial fishermen, and declines in abundance during the 1980s, prompted concern over the health of spawning stocks (Goodyear, 1989). Since the 1980s, fishing regulations have become progressively more restrictive and include size and bag limits. Despite this effort the abundance of subadults in Charleston Harbor, South Carolina, declined through the 1990s (Wenner, 2000).

Several states have studied the feasibility of augmenting natural stocks with hatchery-produced fish (see Seyoum et al., 2000). At present Texas, Florida, and South Carolina are stocking red drum into local waters. Recent efforts in South Carolina have shown that hatchery-produced red drum can substantially increase local populations. The South Carolina Department of Natural Resources released about 1.7 million 40-mm juveniles into Callawassie Creek near Bluffton, over a 3-year period. Of the 1+-year-old fish collected after stocking, about 19% were of hatchery origin (Smith et al., 1999). Such a successful augmentation is, on the one hand, to be applauded and, on the other, a cause for concern. Several authors (see Utter, 1998, and references therein) have noted that hatchery fish are usually produced from a limited number of adults and thus may not possess the full range of genetic variability found in wild stocks. If the progeny of this limited number of adults make a significant contribution to the juvenile pool, it is possible that the genetic resources of wild stock may suffer (Ryman and Laikre, 1991; Tringali and Bert, 1998). The critical parameters for assessing the impact of hatchery production on wild stocks are the effective breeding sizes of both groups and their relative contributions to the juvenile pool (Ryman and Laikre, 1991). It has been concluded from numerical analysis that hatcheries should attempt to maintain effective populations sizes (N_e) of at least 50 to avoid the deleterious effects of inbreeding (Ryman and Laikre, 1991).

While N_e is relatively easy to estimate in captive populations, estimates from natural populations are more difficult and inexact. A variety of approaches have been developed to estimate N_e per generation from gene frequency data (see Waples, 1989), but they often require assumptions that are difficult to justify for many natural populations (see Chakraborty and Neel, 1989). An estimate can be obtained using gene frequency data taken across several year classes (Pollack, 1983; Waples, 1989; Laikre et al., 1998), but this is valid only in so far at the population is semelparous or nearly so. When a population is characterized by overlapping generations, this requires adjustment for longevity and fecundity (Jorde and Ryman, 1995, 1996). Turner et al. (1999) used this approach on mitochondrial DNA data to estimate the effective female population size in red drum. Their estimate was over 14,000 for the entire Gulf of Mexico. This value is substantially less than the long-term effective female population size estimated by Gold et al. (1993a) and much smaller that the estimated census size in this region (approx. 7 million; Nichols, 1988). The estimates are important because they tend to support the notion that there is considerable variance in reproductive success among individual females and is consistent with the sweepstakes hypothesis or Hedgecock effect (Hedgecock, 1994; Ruzzante et al., 1996). A similar conclusion was reached by Chapman et al. (1999a), from a different line of reasoning.

Several aspects of red drum biology are important considerations when evaluating the issue of hatchery enhancement. First, 1- to 3-year-old juveniles are highly residential during their tenure in estuarine systems. Tagging data from Charleston Harbor indicated that about 90% of the juveniles were recaptured within 1 km of the release site (Wenner, 2000). One individual was recaptured 7 times over 311 days within 1 km of its release site (Wenner, 2000). Smith et al. (1999) have shown that hatchery fish will move away from the release site as they grow, but it may take 2 to 3 years for the released fish to make substantial movements within an estuarine system. These data would suggest that the effects of enhancement could be quite localized for some period of time and care should be taken when trying to estimate the proportion of juveniles derived from hatchery stocks. Second, adults are known to spawn in restricted locations at or near the mouths of estuaries (Holt et al., 1985; Wenner, 2000). Spawning activity appears to be restricted to afternoon and evening hours (Holt et al., 1985; Wenner, 2000), and some evidence suggests that peak activity occurs on flooding tides (G. Gilmore, personal communication). This behavior could produce an extreme variance in progeny production among individuals. Spawning on ebbing tides would tend to disperse the eggs into open ocean waters, while spawning on flooding tides would tend to entrain the eggs within the estuary. This could be a contributor to the Hedgecock effect mentioned above.

In this article we examine the distribution of genetic variation in 5 microsatellite loci in red drum. We address two major issues. First is the geographic and temporal

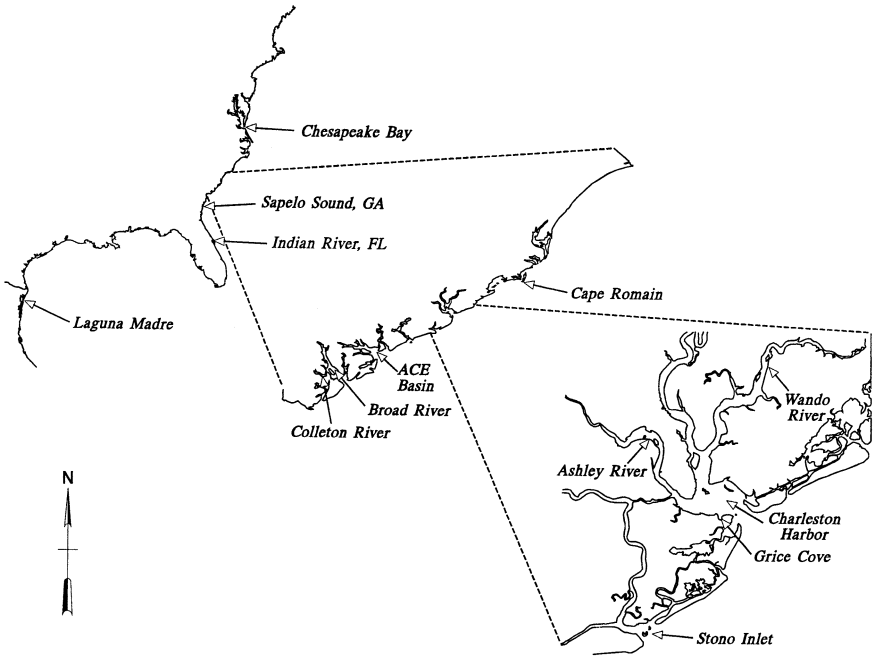

Figure 1. Map showing collecting locations of S. ocellatus in the southeast United States.

partitioning of the genetic variation to characterize population structure. Over the past decade substantial information has been amassed concerning the status of stocks and the genetic relationships among populations (Ramsey and Wakeman, 1987; Gold and Richardson, 1991, 1994; Gold et al., 1993a, 1993b; Chapman et al., 1999a; Turner et al., 1999; Seyoum et al., 2000). For the most part these studies have not found significant population structure except for some weak differentiation between Atlantic and Gulf of Mexico stocks. One notable exception is the report of a genetically distinct aggregation in Mosquito Lagoon, Florida (Gold and Richardson, 1994), which has been questioned by recent data (Seyoum et al., 2000). The second issue is the estimation of N_e using the temporal method described by Waples (1989) for comparison to similar estimates for Gulf of Mexico populations (Turner et al., 1999). The results will be used to examine several management issues including augmentation programs using hatchery-reared individuals.

Materials and Methods

Samples (fin clips or hearts) of 7 year classes (1990–1996) of red drum were taken from major estuarine systems in South Carolina associated with Cape Romain, Charleston Harbor, the Ace Basin, and Calibogue Sound (Figure 1). Samples were collected from multiple locations within the Charleston Harbor Estuary. These included the Ashley River, Charleston Harbor proper, Grice Cove, Stono Inlet, and the upper and lower Wando River. Additional specimens (young-of-the-year) were collected from the Chesapeake Bay, Virginia; Sapelo Sound, Georgia; and the Indian River, Florida. Most specimens taken in the South Carolina estuaries were aged by examining annual rings on the otoliths or scales. In a few cases the age was established by the length of the specimen, which for individuals less than 3 years of age (under 500-mm TL) is accurate more than 90% of the time (C. Wenner, personal communication). Samples from Laguna Madre, Texas, were kindly provided by Dr. John Gold and Ms. Linda Richardson (Texas A&M University).

DNA extraction methods for microsatellite analysis, followed Chapman et al. (1999a). The genomic library for the red drum primers was constructed from fresh heart tissue and screened as described in Ball et al. (1998). We screened approximately 1000 clones with a radioactively labeled $(GT)_{15}$ probe. Forty-eight positive clones were sequenced; of these, 22 had identifiable GT microsatellite repeats, 13 contained appropriate flanking sequences for primer design, and 4 sets of primers were designed (Table 1). Additional primers for RD201 were the gift of Dr. John Gold, and Cne612 has been previously described in Chapman et al. (1999a). Amplified products at Cne612 and Soc029 were separated on native 20 × 25-cm polyacrylamide gels, stained with ethidium bromide, and photographed. A *Hae* III-digested pBluescript plasmid was used as a molecular weight standard. To verify the accuracy of the genotypes at Cne612 and Soc029, selected samples

SEDAR18-RD61

Table 1. Primer and Repeat Sequences, Amplification Conditions, and Results of Amplification of Three Microsatellite Loci in Red Drum

Locus	Primer sequences (5′–3′)	Length (bp)	Repeat sequence	Anneal temp. (°C)
Soc014	GTATGTATTAAGGGCACAAGGTG GATTGCTGCTGGACAGACTG	160	$(GT)_{21}$	50
Soc017	CGCCCGTCTACGTGACAGTATG ATAGCTGCGCATCATTCGGTTG	314	$(GT)_{14}$	50
Soc029	GGCAAATAGTACAGAAAATTA CATGGGATTTCTCTCAGTGACT GGCAGTT	188	$(GT)_{10}$	50

were amplified with ^{32}P-labeled primer and separated on 6% sequencing gels using a ^{35}S-labeled M13 molecular weight standard. For Soc017, Soc014, and RD201, amplifications were conducted with fluorescent labeled primers and separated on an ABI 377 automated sequencer at the Iowa State University DNA sequencing core.

Statistical Analysis

Statistical analyses of the data were preformed using TFPGA Version 1.3 (available at http://www.public.asu.edu/~mmille8/tfpga.htm). Expected heterozygosities were computed using the Levene (1949) correction, and global estimates of F_{1S} were calculated following Weir and Cockerham (1984). Tests for conformity to Hardy-Weinberg equilibrium (Louis and Dempster, 1987; Rousset and Raymond, 1995) were calculated using a Markov chain method (Guo and Thompson, 1992). Tests for allele frequency differences between populations were conducted using Fisher's exact test described in Raymond and Rousset (1995) and Goudet et al. (1996). Pairwise comparisons of all samples at all loci were done and then combined across loci (see Manly, 1985). The default conditions in TFPGA were used for the Markov chain parameters in the tests of Hardy-Weinberg equilibrium and tests for population differentiation. All results were adjusted for multiple simultaneous comparisons using a sequential Bonferroni correction (Rice, 1989). Heirarcha *F* statistics were calculated following Weir and Cockerham (1984). A weighted average over loci was calculated by averaging numerators and denominators separately before taking the ratio (Weir and Cockerham, 1984).

Estimates of the effective number of breeders per year N_b and the effective population size per generation N_e were calculated using the temporal method of Waples (1989) as modified by Jorde and Ryman (1995, 1996) and the demographic factors presented in Turner et al. (1999). The estimates were based only on the combined data from South Carolina. This treatment assumes that there is temporal stability in gene frequencies in the spawning stock over the sampling interval and that the individuals contributing to a year class are a random draw from adult stock regardless of location in South Carolina. The validity of these assumptions will be discussed below. Long-term effective population sizes ($N_{e,t}$) were calculated using the approach of Kimura and Crow (1964), assuming an average mutation rate of 10^{-4}.

Results

Allele frequency distributions for each sampling location and year class are not presented here in the interest of conserving space, but are available upon request from the senior author. In total 19 alleles were observed at RD201, 22 alleles at Soc014, 24 alleles at Soc017, 8 alleles at Cne612, and 6 alleles at Soc029. Observed heterozygosity ranged from about 90% for Soc017 to 38% for Soc029. Significant deviations from Hardy-Weinberg expectation were observed at RD201 in all sampling locations, while few or no significant deviations were observed at Soc017, Cne612, and Soc029 loci (Table 2). About half of the surveyed locations exhibited significant deviations at Soc014 (Table 2). The significant deviations from equilibrium were unilaterally associated with a positive Fis, indicating a heterozygote deficiency in all cases. The most striking feature of the data presented in Table 2 is that significant departures from Hardy-Weinberg equilibrium are noted at most, but not all, loci in tests that pool sampling locations (i.e., Atlantic and South Carolina). Employing the criteria of Chapman et al. (1999b), these tests indicate that the pooled data do not comprise a single panmictic unit and suggest the Wahlund effect as an underlying cause.

The proportions of variance attributable to various hierarchies in the data are presented in Table 3. Overall these data suggest that about equal proportions of the total variance are attributable to difference between Gulf of Mexico and Atlantic, among Atlantic sampling locations, and among locations within South Carolina. There is, however, considerable heterogeneity among the loci in this regard. The Fis estimates indicate that most of the total variance is due to variation within sampling locations.

SEDAR18-RD61

Table 2. Summary Statistics for Five Microsatellite Loci Surveyed in *Sciaenops ocellatus* at the Idicated Locations*

	Locus				
Population	RD201	Soc014	Soc017	Cne612	Soc029
Atlantic					
P	0.0000	0.0000	0.0000	0.0000	0.0168
Heterozygosity unbiased	0.7557	0.8046	0.8589	0.5909	0.6106
Heterozygosity count	0.4932	0.6487	0.7974	0.5980	0.5833
N	736	713	696	781	780
Fis	0.3474	0.1938	0.0716	−0.0120	0.0447
Indian River, Fla.					
P	0.0000	0.8388	0.2547	0.9786	0.3766
Heterozygosity unbiased	0.7484	0.8205	0.8706	0.6216	0.5655
Heterozygosity count	0.4091	0.7000	0.8421	0.6818	0.5455
N	22	20	19	22	22
Fis	0.4534	0.1469	0.0327	−0.0968	0.0354
Sapelo Sound, Ga.					
P	0.0000	0.0012	0.0252	0.9342	0.1814
Heterozygosity unbiased	0.6762	0.7982	0.8587	0.6003	0.6025
Heterozygosity count	0.4394	0.6923	0.8308	0.5821	0.5373
N	66	65	65	67	67
Fis	0.3502	0.1327	0.0325	0.0303	0.1082
All South Carolina					
P	0.0000	0.0000	0.0001	0.0002	0.0376
Heterozygosity	0.7628	0.8022	0.8586	0.5902	0.6194
Heterozygosity	0.4992	0.6380	0.7863	0.5910	0.6028
N	605	587	571	648	647
Fis	0.3456	0.2047	0.0842	−0.0014	0.0268
Colleton River, S.C.					
P	0.0003	0.0000	0.0178	0.8316	0.3073
Heterozygosity unbiased	0.7716	0.7997	0.8659	0.5655	0.6401
Heterozygosity count	0.5030	0.6438	0.8092	0.5949	0.6923
N	165	160	152	195	195
Fis	0.3481	0.1949	0.0655	−0.0520	−0.0815
Broad River, S.C.					
P	0.0000	0.0002	0.0959	0.2159	0.0935
Heterozygosity unbiased	0.7135	0.8034	0.8417	0.5604	0.6151
Heterozygosity count	0.5972	0.5139	0.6944	0.5417	0.5556
N	72	72	72	72	72
Fis	0.1630	0.3603	0.1750	0.0334	0.0967
Ace Basin, S.C.					
Heterozygosity	0.0000	0.0000	0.0578	0.9022	0.1887
Heterozygosity unbiased	0.7594	0.7713	0.8584	0.6083	0.6279
Heterozygosity count	0.4937	0.4792	0.7468	0.6707	0.6341
N	79	72	79	82	82
Fis	0.3499	0.3787	0.1300	−0.1026	−0.0099
Stono Inlet, S.C.					
P	0.0000	0.9404	0.4880	0.2039	0.7882
Heterozygosity unbiased	0.8066	0.7937	0.8628	0.6003	0.6085
Heterozygosity count	0.4694	0.7800	0.8600	0.5636	0.6000

SEDAR18-RD61

Table 2. Continued

	Locus				
Population	RD201	Soc014	Soc017	Cne612	Soc029
N	49	50	50	55	55
Fis	0.4181	0.0173	0.0032	0.0611	0.0140
Charleston Harbor, S.C.					
P	0.0000	0.0004	0.0909	0.4637	0.0019
Heterozygosity unbiased	0.8032	0.8363	0.8216	0.5767	0.5557
Heterozygosity count	0.4038	0.6154	0.7547	0.5926	0.3704
N	52	52	53	54	54
Fis	0.4973	0.2641	0.0814	−0.0276	0.3335
Ashley River, S.C.					
P	0.0000	0.0004	0.0909	0.4637	0.0019
Heterozygosity unbiased	0.7813	0.7368	0.8416	0.5528	0.5913
Heterozygosity count	0.3462	0.4615	0.6800	0.5385	0.6154
N	26	26	25	26	26
Fis	0.5569	0.3736	0.1920	0.0259	−0.0408
Grice Cove, S.C.					
P	0.0000	0.7054	0.1474	0.9847	0.2387
Heterozygosity unbiased	0.7253	0.8224	0.8775	0.5818	0.6090
Heterozygosity count	0.4545	0.8519	0.8333	0.5818	0.6909
N	55	54	42	55	55
Fis	0.3734	−0.0359	0.0504	0.0000	−0.1345
Wando River, S.C.					
P	0.0000	0.0077	0.9084	0.0203	0.9812
Heterozygosity unbiased	0.7458	0.8274	0.8459	0.5791	0.5544
Heterozygosity count	0.5167	0.7407	0.8235	0.6613	0.5410
N	60	54	51	62	61
Fis	0.3072	0.1048	0.0265	−0.1419	0.0242
Cape Romain, S.C.					
P	0.0005	0.5171	0.3222	0.0109	0.0011
Heterozygosity unbiased	0.7367	0.7657	0.8890	0.6772	0.6788
Heterozygosity count	0.5957	0.6596	0.8511	0.4894	0.4894
N	47	47	47	47	47
Fis	0.1914	0.1386	0.0426	0.2773	0.2790
Cape Henry, Va.					
P	0.0005	0.1128	0.8820	0.3249	0.0572
Heterozygosity unbiased	0.7669	0.8136	0.8588	0.5857	0.4914
Heterozygosity count	0.5349	0.7073	0.8780	0.6818	0.3864
N	43	41	41	44	44
Fis	0.3025	0.1307	−0.0224	−0.1641	0.2137
Laguna Madre, Tex.					
P	0.0009	0.2000	0.6151	0.3754	0.2935
Heterozygosity unbiased	0.8228	0.8092	0.8701	0.5212	0.6455
Heterozygosity count	0.6429	0.6923	0.9091	0.4286	0.5714
N	14	13	11	14	14
Fis	0.2186	0.1445	−0.0448	0.1777	0.1148

P values indicate the probability of conformity to Hardy-Weinberg expectations. *N* is the same size.

Table 3. *F* Statistics for Each Locus for the Indicated Hierarchical Structures

Locus	Fit	Gulf vs Atlantic	Among Atlantic	Among South Carolina	Fis
RD201	0.3569	0.0182	0.0179	0.0202	0.3437
Soc014	0.1857	−0.0106	−0.0079	−0.0041	0.1890
Soc017	0.0691	0.000	−0.0014	−0.0002	0.0693
Cne612	0.1146	0.127	0.1181	0.1283	−0.0158
Soc029	0.029	−0.0206	−0.0177	−0.0116	0.0401
All >loci	0.156	0.0212	0.0203	0.0247	0.1346

Table 4. Matrix of Combined Probabilities for Each Pairwise Comparison*

	Indian River, Fla.	Sapelo Sound, Ga.	South Carolina	Chesapeake Bay, Va.
Sapelo Sound, Ga.	0.2686			
South Carolina	0.4830	0.6259		
Chesapeake Bay, Va.	0.5715	0.0083	0.0468	
Laguna Madre, Tex.	0.0001	0.0001	0.0000	0.0004

*All sampling locations in South Carolina were combined. The values indicate the probability that a comparison is statistically identical.

Table 5. Matrix of Combined Probabilities Over All Loci for Each Pairwise Comparison, South Carolina Locations Only*

	Colleton River	Broad River	Ace Basin	Stono Inlet	Charleston Harbor	Ashley River	Grice Cove	Wando River
Broad River	0.0290							
Ace Basin	0.1088	0.0230						
Stono Inlet	0.2530	0.2256	0.0148					
Charleston Harbor	0.0000	0.0011	0.0000	0.0122				
Ashley River	0.0000	0.0021	0.0000	0.0006	0.0977			
Grice Cove	0.1369	0.1644	0.0214	0.0851	0.0013	0.0007		
Wando River	0.0527	0.0552	0.1347	0.2525	0.0044	0.0166	0.4060	
Cape Romain	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000

*The values indicate the probability that a comparison is statistically identical.

Pairwise comparisons of gene frequencies among locations when the South Carolina data were pooled (Table 4) indicated that only samples from Laguna Madre, Texas, were significantly different from other locations. The same test comparing only South Carolina locations (Table 5) indicated that the Charleston Harbor, Ashley River, and Cape Romain samples were statistically different from all other locations. The Charleston Harbor and Ashley River samples were not significantly different. The remaining South Carolina locations appeared to be genetically homogeneous.

Sample sizes for year class comparisons are presented in Table 6, and the analyses of gene frequency data by year classes are presented in Tables 7 and 8. Tests for departure from Hardy-Weinberg equilibrium were significant, or nearly so, at most loci when the year classes were combined. Employing the criteria of Chapman et al. (1999b), these data indicate that the combined year class data were not drawn from a single panmictic unit. However, few tests were significant when conducted on individual year classes, even at RD201, which exhibited consistent deviations when the data were sorted by locations (Table 2). Pairwise

Table 6. Sample Size for Each South Carolina Location by Year Class

Location	1990	1991	1992	1993	1994	1995	Total
Ace Basin	4	5	6	15	25	13	68
Ashley River	1	3	3	5	9	4	25
Broad River					18	54	72
Cape Romain	7	17	8	9			41
Charleston Harbor	9	12	10	8			39
Colleton River				14	103	78	195
Grice Cove					38	17	55
Stono Inlet		1			8	46	55
Wando	3	25	10	16	7	1	62
Totals	24	63	37	67	208	213	612

comparisons of gene frequencies among year classes (Table 8) suggested an interesting pattern. Adjacent year classes tended not to be significantly different (eg., 1990 vs 1991), but as the time interval between year classes increased, the differences tended to increase and become highly significant. The pattern was consistent across all year classes.

Estimates of N_e and N_b using the approach of Waples (1989) are presented in Tables 9 and 10. Prior to 1994 the estimates of N_e were 300 or so, and they increased in 1994 and 1995 as depicted in Figure 2. Some fluctuations are noted at the individual loci, but by and large the data are consistent. The 95% confidence intervals suggest that these estimates are accurate to within a factor of 2. The $N_{e,t}$ ranged from about 3000 to 15,000 and averaged 8205 across all loci.

Discussion

The data presented here are relevant to at least 4 issues concerning the population genetics, management policy, and conservation of red drum: the population structure of the species in both time and space, the number of breeders responsible for each year class, the potential impact of hatchery augmentation on wild stocks, and the prospect that variance in reproductive output (the sweepstakes hypothesis) may be evident in this species. While these issues are interrelated, they will be dealt with independently in the interest of clarity.

Population Structure

Previous studies of the population structure of red drum have in the main indicated that there is limited differentiation among red drum populations (Ramsey and Wakeman, 1987; Gold and Richardson, 1991, 1994; Gold et al. 1993a, 1993b; Chapman et al., 1999a; Turner et al., 1999; Seyoum et al., 2000). What differentiation exists appears to be consistent with a model of isolation by geographic distance, and for the most part populations of red drum should be considered as a single management unit. The data presented here strongly support this conclusion, as the Laguna Madre samples were statistically different from Atlantic samples, while comparisons along the Atlantic Coast were not statistically different (see Table 3). The exception to this generality in the literature is the report by Gold and Richardson (1993a), which suggested that red drum from Mosquito Lagoon, Florida, were distinct from other locations based on allozyme data. The data in Table 4 indicate that the report of Gold and Richardson may not be all that exceptional. For example, the statistical difference noted between the Ashley River and Charleston Harbor samples compared to other samples in the Charleston Estuary indicates that fine scale differences may indeed be found in red drum populations. The differences between Cape Romain and other South Carolina populations simply reinforce this conclusion. It is our view that the genetic distinction of the Mosquito Lagoon population is not an error (at least not one made by Gold and Richardson, 1993a), but a real phenomenon that stems from the species biology.

The temporal variation noted in the present study contrasts sharply with the observations of mtDNA variability (Gold and Richardson, 1993b; Turner et al., 1999) and microsatellite variation (Turner et al., 2001) of populations in the Gulf of Mexico. The data presented here strongly indicate significant differences among year classes. The discrepancies between these data are not as polarized at they may seem. The data generated from the Gold laboratory are based primarily upon 4-year classes spanning 1986 to 1989. The data presented here are based upon 6 year classes spanning the 1990 to 1995. The data in Table 8 clearly indicate that had we confined our sampling to any 4 year classes, the support for significant year class variation would have been problematic at best. It is only with the inclusion of all 6 year classes that the trend in the data becomes apparent. Thus the discrepancy between our data and those of the Gold laboratory may well be due to the time scale over which the data were collected. In addition, the present data were collected during a period of substantial decline in juvenile red drum populations in South Carolina (Wenner et al., 2000). These reductions could well produce bottlenecks that would be reflected in genetic

Table 7. Tests for Conformity to Hardy-Weinberg by Year Class

	RD201	Soc014	Soc017	Cne612	Soc029
All year classes	0.0000	0.0333	0.5568	0.0001	0.0533
1990	0.7411	0.5229	0.5634	0.2526	0.0781
1991	0.0195	0.4472	0.5306	0.0729	0.0736
1992	0.2006	0.9476	0.0021	0.1111	0.0696
1993	0.3313	0.4646	0.8722	0.0232	0.0650
1994	0.0000	0.1308	0.9490	0.5514	0.2045
1995	0.0578	0.5600	0.4079	0.0196	0.4091

Table 8. Pairwise Comparison of Gene Frequencies Across All Loci by Year Class*

	1990	1991	1992	1993	1994
1991	0.3191	—			
1992	0.2060	0.0736	—		
1993	0.9624	0.0338	0.0523	—	
1994	0.0576	0.0000	0.0058	0.1444	—
1995	0.0276	0.0001	0.0012	0.0737	0.1750

*Values indicate the probability that gene frequencies are identical.

differentiation among year classes. In effect our data may present a somewhat biased view of the ''natural'' dynamics influenced by population declines. Nonetheless, the genetic differences noted between year classes are clear and unambiguous.

Overall, the data presented here are consistent with previous studies of red drum in that significant differences were found between Gulf of Mexico and Atlantic populations, but limited divergence was apparent along the Atlantic Coast on a broad scale. Fine scale differences were noted among locations in South Carolina; however, these differences may have been due to year class variation rather than geography. Several lines of evidence support this conclusion. The significant departures from Hardy-Weinberg equilibrium noted at most loci in the combined South Carolina locations (Table 2) were not eliminated at RD201 and Soc014 when the data were decomposed into individual locations (Table 2). Normally we would ascribe such deviations to the presence of null alleles. However, when the data were decomposed by year class, the departures were largely eliminated (Table 7). The progressive differentiation of year classes as they become more separated in time also suggests that temporal phenomena are more important than spatial differences per se.

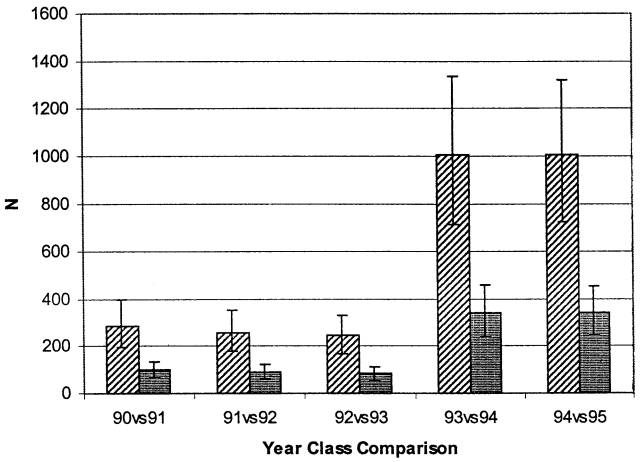

Figure 2. Estimates of effective population size (N_e diagonal) and effective number of breeders (N_b horizontal) in *S. ocellatus* taken from South Carolina waters. Bars indicate 95% confidence limits. Horizontal axis indicates year classes compared. See text for details.

Effective Population Sizes

The estimates of N_e and N_b presented in Tables 9 and 10 suggest that the population sizes of red drum contributing to juveniles in South Carolina were relatively small. It should be noted that N_e and N_b differed only in the scaling factor (17.28/5.92 = 2.9189) used to adjust for overlapping generations (see Turner et al., 1999). These estimates were an order of magnitude smaller than estimates of effective female population sizes in the Gulf of Mexico (Turner et al., 1999), and the 95% confidence intervals suggest that the differences between Gulf of Mexico and Atlantic samples are statistically significant. Several factors could contribute to this difference. First, the correction factors were based, in part, upon age-specific survivorships obtained from juveniles in the Gulf of Mexico (Green et al., 1985), which are substantially higher than those reported in the Atlantic (Latour, 2000). This would tend to depress the estimate of

Table 9. Effective Population Sizes (N_e) Calculated Using the Waples (1989) Method and Long-term Effective Population Calculated Using the Methods of Crow and Kimura (1964)*

Locus	N_e	1990 vs 1991	1991 vs 1992	1992 vs 1993	1993 vs 1994	1994 vs 1995	Over all year classes	Long-term
RD201	Mean	107.95	−60.83	729.91	−172.29	250.92	171.1307	8039.63
	Upper	202.93	−114.36	1305.55	−291.48	412.39	303.0076	
	Lower	42.54	−23.97	317.88	−83.40	127.99	76.20744	
Soc014	Mean	59.21	107.36	35.03	496.81	7175.27	1574.737	10139.03
	Upper	103.75	181.64	61.37	840.49	11792.66	2595.98	
	Lower	26.84	51.97	15.87	240.49	3660.13	799.0594	
Soc017	Mean	62.80	698.53	8402.86	−201.70	644.21	1921.341	15180.34
	Upper	108.03	1164.02	14454.24	−331.50	1033.22	3285.602	
	Lower	29.48	347.59	3943.72	−102.89	343.02	912.1852	
Cne612	Mean	67519.74	46.08	−39949.84	99.04	493.38	5641.678	3600.54
	Upper	130881.21	89.32	−80282.78	191.97	956.38	10367.22	
	Lower	23063.33	15.74	−12369.32	33.83	168.53	2182.421	
Soc029	Mean	−347.37	−118.46	308.62	−1195.22	−404.17	−351.32	4068.58
	Upper	−823.93	−280.97	234.10	−2646.33	−894.87	−882.40	
	Lower	−61.72	−21.05	70.70	−273.82	−92.59	−75.69	
All loci	Mean	288.87	260.21	244.33	1003.07	1003.72	560.04	8205.62
	Upper	397.07	353.30	333.72	1337.03	1320.17	748.25	
	Lower	195.56	179.43	166.99	710.51	724.61	395.42	

*See text for details. Upper and lower indicate 95% confidence boundaries.

N_e relative to N_b and may account for some of the differences. An additional factor that may account for some of the difference is that the samples used Turner et al. (1999) were taken from the 1986–1989 year classes, while those employed here were taken from 1990–1995 year classes. As the population in South Carolina declined over this period (Wenner, 2000), some of the difference may be due to this factor as well. Finally, these differences could be taken at face value and indicate that N_e and N_b are simply smaller in the Atlantic than in the Gulf of Mexico.

An interesting feature of the estimates of N_e and N_b is the increase noted in 1993 versus 1994 and 1994 versus 1995 estimates versus previous years (Tables 9 and 10; Figure 2). While it is premature to suggest that these data indicate a general trend, they do coincide with more restrictive management policy implemented in the late 1980s. By 1989 size limits were fully implemented in South Carolina to protect juvenile red drum for at least the first year of life. The intent was to increase escapement of juvenile individuals and increase the spawning stock. As the species requires 3 to 4 years to reach sexual maturity, the apparent increase in N_e and N_b might be taken to reflect the impact of the size limit policy.

The basic assumption under which the estimates of N_e and N_b were derived was that the year classes were drawn from a single gene pool. This would imply that the gene frequencies in the adults were stable over time and the adults spawning in a particular estuary were a random draw from the Atlantic Coast stock. These could be addressed, at least in principle, by examining gene frequency distributions in spawning aggregations. However, we do not have the requisite samples, and obtaining sufficient numbers from actual spawning locations is not practical at this time because only 2 spawning locations are known in South Carolina (C. Wenner, personal communication), and they are both subject to strong currents that preclude the use of nets. Gold and Turner (2002) have suggested that the neighborhood size of red drum was between 700 and 900 km which is much larger that the area covered by the South Carolina collections. Thus the assumption that the year classes were drawn from a single gene pool is plausible. The lack of significant gene frequency differences among sam-

Table 10. Effective Number of Breeders (N_b) Calculated Using the Waples (1989) Method*

Locus	N_b	1990 vs 1991	1991 vs 1992	1992 vs 1993	1993 vs 1994	1994 vs 1995
RD201	Mean	36.98	−20.84	250.06	−59.03	85.96
	Upper	69.52	−39.18	447.27	−99.86	141.28
	Lower	14.57	−8.21	108.90	−28.57	43.85
Soc014	Mean	20.29	36.78	12.00	170.20	2458.20
	Upper	35.54	62.23	21.03	287.94	4040.08
	Lower	9.19	17.80	5.44	82.39	1253.93
Soc017	Mean	21.52	239.31	2878.76	−69.10	220.70
	Upper	37.01	398.79	4951.91	−113.57	353.97
	Lower	10.10	119.08	1351.09	−35.25	117.52
Cne612	Mean	23131.76	15.79	−13686.52	33.93	169.03
	Upper	44838.93	30.60	−27504.29	65.77	327.65
	Lower	7901.33	5.39	−4237.64	11.59	57.74
Soc029	Mean	−119.01	−40.58	105.73	−409.47	−138.47
	Upper	−282.27	−96.26	80.20	−906.61	−306.58
	Lower	−21.15	−7.21	24.22	−93.81	−31.72
All loci	Mean	98.96	89.15	83.71	343.65	343.87
	Upper	136.03	121.04	114.33	458.06	452.28
	Lower	67.00	61.47	57.21	243.41	248.25

*See text for details. Upper and lower indicate 95% confidence boundaries.

ples taken along the Atlantic Coast would also tend to support the assumption under which N_e and N_b were estimated.

The estimates of $N_{e,t}$ were an order of magnitude larger than contemporary estimates of N_e. This could be taken at face value and indicate N_e in red drum has declined, consistent with declines in the census size noted over the past 2 decades (Goodyear, 1989; Wenner, 2000). However, $N_{e,t}$ estimates depend upon the assumed mutation rates (see Kimura and Crow, 1964), which are not known with any precision and are likely to vary among loci. Thus estimates of $N_{e,t}$ should be viewed with suspicion and not used to support any strong conclusions.

Impact of Hatchery Augmentation

Tringali and Bert (1998) have suggested that red drum are in no apparent danger of genetic swamping from current augmentation programs. This conclusion was based upon $N_{e,t}$ = 372,000 and the value was attributed to Gold et al. (1993a). The number does not appear in Gold et al. (1993a) and seems to be an inflation of the estimate of effective female population size of 93,000 by a factor of 4 to compensate for the differences between mtDNA and nuclear genes. This discrepancy aside, $N_{e,t}$ is not germane to the issue of stock enhancement as it reflects the genetic diversity accumulated over the life span of the species. The genetic diversity of long-lived, iteroparous species such as red drum does not change rapidly as a result of population bottlenecks (see Nei, 1978), and the estimates of $N_{e,t}$ derived from measures of diversity may well be inflated due to this lag. The important consideration for the effects of augmentation programs is the contemporary population size, which is reflected in the Waples (1989) approach. These estimates for red drum (Turner et al., 1999, and Table 8) are much smaller than the value used by Tringali and Bert (1998).

The impact of augmentation programs on wild populations depends on the effective sizes of the wild (N_w) and captive (N_c) populations and their relative contributions to subsequent generations (Ryman and Laikre, 1991). When $N_w \gg N_c$ the impact of stocking is a unilateral depression in N_e (Tringali and Bert, 1998). However, when $N_c > N_w\, x / (2-x)$, where x is the contribution of the captive population to the wild stock, the augmentation program actually increases N_e. In the present case estimates of population sizes are all less than 1000, the contribution of hatchery-reared individuals to wild stocks are less than 20% (Smith et al.,

1999), and the hatchery population size is on the order of 50 per generation. Thus the augmentation program is not likely to have a serious impact on N_e.

Sweepstakes Hypothesis

The high fecundity and early mortality that characterize many aquatic species could lead to a high variance in reproductive success among individuals and lower N_e (Hedgecock, 1994). This sweepstakes hypothesis has been suggested as an explanation for temporal variation in gene frequencies noted between year classes in some species (Hedgecock et al., 1992; Ruzzante et al., 1996). In a previous paper (Chapman et al., 1999a), it was suggested that the sweepstakes was a plausible explanation for the differences noted in population structure between red drum, spotted sea trout, and weakfish. Gold and Turner (2002) found no evidence of genetic difference between year classes of red drum from the Gulf of Mexico and thus no support for the sweepstakes hypothesis. The present data did support significant differences among year classes and conformity to Hardy-Weinberg equilibrium within year classes, which is consistent with the expectations for populations experiencing a sweepstakes effect.

We believe that the dichotomy between the data of Gold et al. (1999) and Gold and Turner (2002) and our own concerning the sweepstakes hypothesis lies in the differences in sample sizes relative to N_e. In those studies the sample sizes were on the order of 1300 and about an order of magnitude smaller than the estimates of N_e. In the present data the samples sizes and estimates of N_e are about the same. In other words the differences are due, in our view, to the fact that Gulf of Mexico populations of red drum are larger than those in the Atlantic and larger samples sizes are required to detect differences between year classes. The differences are not the result of overall dynamics of red drum populations. Gold and Turner (2002) recognized this possibility.

Management Implications

With regard to implications of the current data for management, 2 features of the data bear closer scrutiny. One is the magnitude of estimates of N_e and the confidence we can place in these estimates. The other is the genetic impact of hatchery programs on the wild population. Again, these issues cannot be divorced from each other, as the former impacts the later and vice versa.

A critical question at this point is how much confidence can we actually place in the estimates of N_e? Mathematical waffling aside, do the numbers bear any resemblance to reality? The answers are important because the numbers are disturbingly small and point to a fragile resource. We are of the view that these estimates are not far from the truth. As Smith et al. (1999) have demonstrated, hatchery augmentation can make a significant contribution to wild stocks. In that study 1.7 million fingerlings stocked into the Callawassie Creek comprised nearly 20% of the juveniles captured the next year. This compares to the many million eggs that could be produced by a large female in a single spawning season (Wenner et al., 2000). Thus it is within reason that a small number of individuals could produce the bulk of offspring in any given year and result in small effective population sizes. In addition, Turner et al. (2001) have recently demonstrated that the temporal method of Waples (1989) tends to overestimate N_e when the actual value and sample sizes are near those presented here. We suspect, therefore, that the real values are somewhat less than our estimates. Even if our estimates are low by an order of magnitude and more in line with those from the Gulf of Mexico (Turner et al., 1999), they underscore the need for the strict conservation measures that have been adopted for this species.

While estimates of N_e are important for understanding the population genetics of a species, the estimate of N_b is more significant for management. N_b is an assessment of the spawning population with minimal lag and unencumbered by estimates of longevity and fecundity, which are difficult to measure under any circumstances.. Monitoring of red drum juveniles from 1996 to the present would add measurably to understanding of the population and allow us to track the effectiveness of current management. It is critical to determine if the trend suggested by Figure 2 is real or simply a random fluctuation.

An important consideration for the impact of augmentation programs is the implicit assumption that measures of genetic variation extracted from molecular techniques reflect the variation in quantitative traits that are important for the survival of the species (see Tringali and Bert, 1998). The assumption has rarely been tested, but the available theoretical and empirical data indicate that the correlation is weak to nonexistent (Pfrender et al., 2000; Reed and Frankham, 2001). Theoretical work has shown that in small populations the variation of quantitative traits may be above detection thresholds while heterozygosity at most loci may be virtually nonexistent (Pfrender et al.,

2000). This conclusion stems from the fact that quantitative traits are, by definition, important to the fitness of the individual and not neutral markers subject only to the whims of mutation, migration, and drift. Thus small population size per se does not necessarily dictate a reduction in variation in quantitative traits. Microsatellites have been the focus of population genetics research in large part for their presumptive neutrality, and thus allow estimates of population parameters uncomplicated by the effects of selection (Wright and Bentzen, 1994). Reed and Frankham (2001) demonstrated a weak correlation between molecular and quantitative measure of variation in 71 data sets. They concluded that molecular measures of variation are limited in their ability to predict quantitative variation (Reed and Frankham, 2001). Thus the linkage of molecular measure of variation and quantitative variability lacks theoretical and empirical support.

There is a serious concern relevant to small population size that has largely been ignored by conservation geneticists. The accumulation of deleterious recessives, as a result of inbreeding in small populations, is a well-known phenomenon. For the moment the heterozygosity estimates for red drum do not suggest any cause for concern on this account. We do not know of any deleterious recessives in red drum, but they almost certainly exist, as they are found in all species that have been intensively studied (e.g., *Drosophila*, mice, and humans). The accumulation of deleterious recessives in small populations may be a more serious threat than the loss of quantitative variation, but we have no empirical data that bear on the issue.

The conclusions presented here are based mainly upon the data collected from South Carolina waters and may not be applicable to other regions. The impact of augmentation programs may be substantially different in the Gulf of Mexico, where N_e is large. It is also our opinion, and that of the hatchery personnel who do the work, that augmentation programs are no substitute for effective management of this species and the ecosystems upon which it depends.

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