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Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean

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Abstract. Variation in mitochondrial (mt)DNA was examined among 473 red drum (*Sciaenops ocellatus*) sampled in 1988 and 1989 from nearshore localities in the northern Gulf of Mexico (Gulf) and the Atlantic coast of the southeastern United States (Atlantic). Data were combined with those from a previous study to generate a total of 871 individuals sampled from 11 localities in the Gulf and 5 localities in the Atlantic. Individuals assayed were from the 1986 and 1987 year-classes. A total of 118 composite mtDNA genotypes (haplotypes) was found. The percentage nucleotide sequence divergence among the 118 haplotypes ranged from 0.184 to 1.913, with a mean ($\pm$SE) of 0.878 $\pm$ 0.004. MtDNA nucleon diversities and intrapopulational nucleotide-sequence divergence values were similar over all Gulf and Atlantic localities, and were high relative to most fish species surveyed to date. These data indicate that the perceived decline in red drum abundance appears not to have affected the genetic variability base of the species. Significant heterogeneity in the frequencies of at least four haplotypes was detected between pooled samples from the Gulf vs pooled samples from the Atlantic. No heterogeneity was found among localities from the Gulf or localities from the Atlantic. High levels of gene flow among all localities were inferred from F_{ST} values (a measure of the variance in mtDNA haplotype frequencies) and from Slatkin's qualitative and quantitative analyses. Parsimony and phenetic analyses revealed no strong evidence for phylogeographic cohesion of localities, although there was weak support for cohesion of four of five localities from the Atlantic. These data indicate that the red drum population is subdivided, with weakly differentiated subpopulations (stocks) occurring in the northern Gulf and along the Atlantic coast of southeastern USA. Spatial autocorrelation analysis and heterogeneity tests of haplotype frequencies among regions within the Gulf supported the hypothesis of increased gene flow among neighboring localities; i.e., migration of individuals within the Gulf may be inversely related to geographic distance from an estuary or bay of natal origin. Estimates of evolutionary effective female-population size indicate that the red drum subpopulations may be large.

Introduction

The red drum (*Sciaenops ocellatus*) is an estuarine-dependent sciaenid fish distributed throughout the northern Gulf of Mexico (Gulf) and along the Atlantic coast (Atlantic) of the southeastern United States (Lux and Mahoney 1969, Matlock 1984, Reagan 1985). The species supports an important fishery along coastal areas of the southeastern U.S. (Matlock 1984, Mercer 1984, Swingle 1987), and considerable effort has been expended recently to domesticate the species for aquaculture (Chamberlain et al. 1987). Declines in red drum abundance and recruitment (Matlock 1984, Reagan 1985, Goodyear 1989) and apparently high rates of annual mortality of individuals in the younger age classes (Murphy and Taylor 1990) have led to relatively strict management measures designed to reduce red drum growth and recruitment-overfishing (Swingle et al. 1984, Goodyear 1989). Two questions of importance to present and future management of red drum are: (i) do discrete subpopulations (stocks) exist either within the Gulf or between the Gulf and Atlantic? and (ii) have declines in abundance and recruitment affected the genetic diversity of the species (McIlwain et al. 1986)? Benefits of obtaining answers to these questions relate, in part, to whether the red drum fishery could (or should) be managed on subregional bases and whether subregions are differentially impacted.

Genetic studies utilizing nuclear-gene (allozyme) loci have indicated the possible existence of population subdivision among red drum from the Gulf. Ramsey and Wakeman (1987) and Bohlmeyer and Gold (1991) found significant heterogeneity in nuclear-gene allele frequencies among red drum taken from different localities in the Gulf, and allelic variation at a locus for adenosine deaminase appeared to follow a clinal or geographic pattern (Bohlmeyer and Gold 1991). Gold and Richardson (1991), however, found no heterogeneity in mtDNA haplotype frequencies among the red drum from the Gulf examined by Bohlmeyer and Gold. Gold et al. (1993), using the same polymorphic (allozyme) loci as Bohlmeyer and Gold, examined an additional 456 red drum from the Gulf and found no heterogeneity in allele frequency at

any locus. They suggested that the heterogeneity among red drum from the Gulf detected by Bohlmeyer and Gold was possibly due to insufficient numbers sampled or sampling error. Both allozyme and mtDNA data indicate genetic heterogeneity between red drum from the Gulf and red drum from the Atlantic (Bohlmeyer and Gold 1991, Gold and Richardson 1991, Gold et al. 1993).

Non-genetic data bearing on red drum population structure in the Gulf are contradictory. Several aspects of red drum biology and life-history suggest that individual dispersal could be extensive, thereby minimizing spatial differentiation. Red drum spawn near the mouths of bays or estuaries (Matlock 1984, 1987) and eggs, larvae, or juveniles could be transported to adjacent bay or estuarine localities by oceanic currents (Lyczkoski-Schultz et al. 1988). Moreover, although larvae and juveniles appear to remain in the bays and estuaries, sexually-mature adults move into deeper waters prior to spawning and are capable of forming large, offshore schools that can migrate extensively (Overstreet 1983, Matlock 1984, 1987, Swingle et al. 1984). Tagging studies of subadult red drum, however, have indicated that movement among nearshore localities is limited (Adkins et al. 1979, Matlock and Weaver 1979, Osburn et al. 1982).

The purposes of the present study were to (i) further test the hypothesis that red drum in the Gulf are spatially subdivided, (ii) test for temporal heterogeneity in mtDNA haplotype frequencies between samples from different year-classes, and (iii) estimate levels of genetic variation within and among localities from both the Gulf and the Atlantic. Data on restriction-site variation in the mtDNA molecules of a total of 871 red drum from two year-classes in the Gulf and Atlantic are presented. Most of the red drum samples were from the Gulf and included three localities from the northwestern Gulf not surveyed by Gold and Richardson (1991). Estimates of evolutionary effective female-population sizes for red drum from the Gulf and Atlantic are also presented.

Materials and methods

White muscle, kidney, and heart tissues were removed in 1988 and 1989 from 437 and 36 specimens of *Sciaenops ocellatus* from the Gulf and Atlantic, respectively, and placed in liquid nitrogen for transport to the laboratory, where they were stored at −80 °C. Specimens were obtained by gill nets, trammel nets, haul seines, and hook and line. Collection localities and the number of individuals taken at each locality by year-class are given in Fig. 1 and Table 1. Ages of all but yearling (Age zero) individuals (i.e., specimens <300 mm total length) were determined from annuli on otoliths using methods described in Bumguardner (1991). The first annulus was considered to have formed 14 to 15 mo after hatching (Bumguardner 1991).

Details regarding the assay of mtDNAs of individual fish may be found in Gold and Richardson (1991). Thirteen restriction endonucleases were used to digest mtDNA molecules: *Bam*HI, *Bcl*I, *Eco*RV, *Hin*dIII, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Stu*I, *Xba*I, and *Xmn*I. Lambda DNA digested with *Hin*dIII was used as a molecular weight marker on each gel. MtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances.

All restriction sites detected in red drum mtDNA have been mapped using single- and double-digestions (Schmidt and Gold 1992). The total number of mtDNA restriction sites surveyed in this study was 104. The numbers of restriction sites surveyed per restriction enzyme were: *Bam*HI (2), *Bcl*I (8), *Eco*RV (7), *Hin*dIII (6), *Nco*I (11), *Nsi*I (5), *Pst*I (6), *Pvu*II (7), *Sca*I (13), *Spe*I (11), *Stu*I (10), *Xba*I (9), and *Xmn*I (9). Homology of fragment patterns from single digestions of mtDNA molecules was tested by multiple side-by-side comparisons of all variant patterns produced by each enzyme. Except for fragment patterns where only a single band of greater than 15 kilobases (kb) was observed, homology was assumed if fragments were the same size in side-by-side comparisons. All variant patterns exhibiting only a single band of >15 kb were tested for homology using double digestions with the enzyme *Bam*HI as described by Gold and Richardson (1991). Restriction sites for the enzyme *Pst*I were inferred from double digestions with *Bam*HI as described by Gold and Richardson.

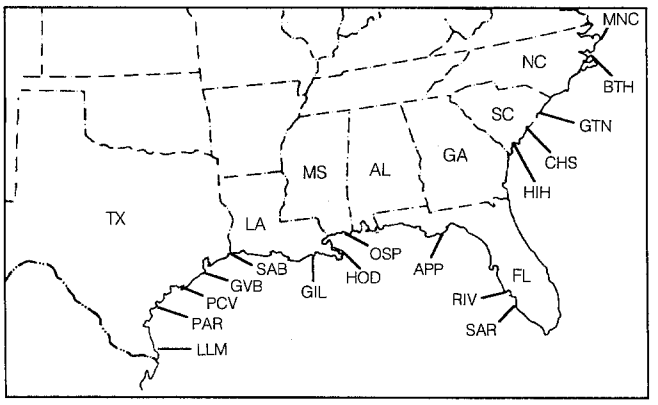

Fig. 1. Sample localities of *Sciaenops ocellatus* examined in present study. Acronyms for localities and site descriptions are given in Table 1. State abbreviations = TX, Texas; LA, Louisiana; MS, Mississippi; AL, Alabama; GA, Georgia; FL, Florida; SC, South Carolina; NC, North Carolina

Nucleon diversity values within geographic localities and among all red drum examined were calculated following Nei and Tajima (1981). Significance testing of haplotype frequencies among localities (spatial) and among age classes (temporal) was carried out using (i) the *V*-statistic on arcsine square-root-transformed haplotype frequencies (DeSalle et al. 1987), (ii) a randomization (bootstrap) procedure developed by Roff and Bentzen (1989), and (iii) the *G*-statistic (Sokal and Rohlf 1969) employing BIOM-PC (Rohlf 1983). Bootstrapping employed the MONTE program in REAP ("restriction enzyme analysis package"), a statistical package for analyses of mtDNA data (McElroy et al. 1992). Significance levels for multiple tests performed simultaneously were adjusted to P/n, where P was the significance probability associated with a specific test and n was the number of independent tests performed simultaneously (Cooper 1968). F_{ST} values were calculated using formulae in Weir and Cockerham (1984) and computer programs described in Weir (1990). Estimates of gene flow ($N_e m_f$), the effective number of female migrants per generation, were calculated using Wright's (1943) island model modified for mtDNA, where $F_{ST} \simeq 1/(2N_e m_f + 1)$. Average levels of gene flow and $N_e m_f$ values among geographic localities were also estimated using Slatkin's (1981, 1985) qualitative and quantitative methods, respectively. For Slatkin's quantitative method, average sample sizes were calculated as harmonic means.

A restriction-site presence/absence (binary) matrix of mtDNA haplotypes for estimation of nucleotide-sequence divergence values and for parsimony analyses was constructed using the GENERATE program in REAP (McElroy et al. 1992). Restriction-site matrices of geographic localities were constructed using the mtDNA haplotype matrix. For each locality, a restriction site was coded as present (Code 1) if it occurred in the locality, or absent (Code 0) if it did not. Nucleotide-sequence divergence values among mtDNA haplotypes were estimated using the site approach of Nei and Li (1979) and the DSE program in REAP (McElroy et al. 1992). Nucleotide-sequence

Table 1. *Sciaenops ocellatus.* Acronyms of collection localities used throughout paper, and number of red drum collected in present study; "total" column shows present material plus that studied by Gold and Richardson (1991)

Locality	Year-class	No. of individuals	
		present study	total
Gulf of Mexico	1986	134	392
	1987	303	303
LLM: Lower Laguna Madre, Texas	1986	18	18
	1987	21	21
ULM: Upper Laguna Madre, Texas	1986	0	0
	1987	2	2
PAR: Redfish Bay, Texas	1986	8	17
	1987	21	21
PCV: Pass Cavallo, Texas	1986	13	13
	1987	18	18
GVB: West Bay, Texas	1986	0	32
	1987	36	36
SAB: Sabine Pass, Texas	1986	25	25
	1987	18	18
GIL: Grand Isle, Louisiana	1986	0	43
	1987	47	47
HOD: Black Bay, Louisiana	1986	0	20
OSP: Biloxi Bay, Mississippi	1986	26	83
	1987	34	34
APP: Apalachicola Bay, Florida	1986	6	30
	1987	37	37
RIV: Rivera Bay, Florida	1986	0	24
	1987	45	45
SAR: Sarasota Bay, Florida	1986	38	87
	1987	24	24
Atlantic Ocean	1986	0	140
	1987	36	36
HIH: Calibogue Sound, South Carolina	1986	0	50
CHS: Charleston Bay, South Carolina	1986	0	34
	1987	32	32
GTN: North Inlet, South Carolina	1986	0	18
BTH: Palmico River, North Carolina	1986	0	23
	1987	4	4
MNC: Oregon Inlet, North Carolina	1986	0	15

divergence values within and among localities were generated using the intrapopulational and uncorrected interpopulational nucleotide-sequence diversity indices of Nei and Tajima (1981), respectively.

Parsimony analysis of the restriction-site presence/absence matrix of mtDNA haplotypes was used to produce a "gene tree" (Avise 1989) and employed the MULPARS option in Version 3.0 of the "phylogenetic analysis using parsimony" (PAUP) program of Swofford (1990). Parsimony analyses of the binary matrices representing localities employed both PAUP and the BOOT program in Version 2.9 of the "phylogeny inference package" (PHYLIP) of Felsenstein (1987). For bootstrapping, two different random-number seeds were used and each was run with 50 replacements. Estimates of confidence for individual branches were summed from the two BOOT runs. All autapomorphies and symplesiomorphies were removed from binary matrices using the REDUCE program in REAP (McElroy et al. 1992). Phenetic analysis of the matrix of nucleotide-sequence divergence values among haplotypes and of the matrices of interpopulational nucleotide diversities among localities was carried out using UPGMA (unweighted pair-group method using arithmetic averages) clustering (Sneath and Sokal 1973). The program used for UPGMA clustering also computed a standard error for each node in the phenogram using equations in Nei et al. (1985).

Spatial autocorrelation analysis of frequencies of common haplotypes was carried out using the "spatial autocorrelation analysis program" (SAAP) of Wartenberg (1989) following recommendations in Sokal and Oden (1978a, b). These analyses involve computation of autocorrelation coefficients (Moran's I values) as a function of geographic distance between pairs of localities, and summarize (through correlograms) the patterns of geographic variation exhibited by the response surface (geographic distance) of any given variable (haplotype frequencies). The autocorrelation analyses were restricted to the 11 geographic localities sampled from the Gulf, with individuals from both year classes (1986 and 1987) pooled at each locality. In order to minimize "noise" generated by low-frequency haplotypes, autocorrelation coefficients were generated from each of two data sets: the first included only haplotypes that were found in 4 or more individuals (33 haplotypes total); the second included only haplotypes that were found in 10 or more individuals (17 haplotypes total). Five distance classes were used in each of two SAAP runs for both data sets: the first SAAP run employed equal numbers of pairwise comparisons (11 each) in each distance class; the second employed equal geographic distances between distance classes. The number of pairwise comparisons by distance class (1 to 5) in the latter was 8, 17, 4, 7, and 9.

Results

Population subdivision

MtDNA fragment patterns from single digestions with the 13 restriction enzymes generated 118 composite genotypes (mtDNA haplotypes) among the 871 specimens of *Sciaenops ocellatus* surveyed. No evidence for mtDNA size variation was observed. Two individuals [one from the 1986 year-class from the Pamlico River, NC (BTH) and one from the 1987 year-class from Charleston Bay, SC (CHS)] were found to be heteroplasmic for single restriction-enzyme sites (Gold and Richardson 1990). The two heteroplasmic individuals were excluded from all further analyses. (A listing of the 118 mtDNA haplotypes, including fragment patterns of the 13 enzymes for each haplotype and the geographic distribution of individual haplotypes, may be obtained upon request from the first author.) Nucleotide-sequence divergence (in percentage) among the 118 haplotypes ranged from 0.184 to 1.913, with a mean ($\pm$SE) of 0.878 ± 0.004.

MtDNA nucleon and intrapopulational nucleotide-sequence diversities are shown in Table 2 for (i) all Gulf localities by year-class, (ii) the 1986 and 1987 year-classes in the Gulf, and (iii) by region (i.e., Gulf and Atlantic). Nucleon diversities among localities in the Gulf ranged from 0.922 to 0.987, with values for the two year-classes being essentially identical. Nucleon diversities among localities in the Atlantic were slightly lower (Gold and Richardson 1991), a difference indicated by overall nucleon diversities among individuals from the Gulf versus

Table 2. *Sciaenops ocellatus*. Mitochondrial DNA nucleon and intrapopulational nucleotide-sequence diversities among localities from Gulf of Mexico. Locality acronyms as in Table 1. Standard deviations are used instead of standard errors because of large number of pairwise comparisons used to generate mean values

Year-class	No. of individuals	No. of haplotypes	Nucleon diversity	Nucleotide sequence diversity (% ± SD)
LLM-86	18	16	0.987	0.632 ± 0.282
LLM-87	21	14	0.952	0.590 ± 0.270
PAR-86	17	13	0.971	0.650 ± 0.312
PAR-87	21	14	0.948	0.658 ± 0.335
PCV-86	13	11	0.974	0.661 ± 0.289
PCV-87	18	14	0.961	0.704 ± 0.363
GVB-86	32	19	0.948	0.526 ± 0.273
GVB-87	36	21	0.960	0.559 ± 0.308
SAB-86	25	17	0.953	0.638 ± 0.315
SAB-87	18	12	0.941	0.470 ± 0.265
GIL-86	43	24	0.966	0.620 ± 0.318
GIL-87	47	22	0.922	0.608 ± 0.341
OSP-86	83	29	0.936	0.493 ± 0.278
OSP-87	34	21	0.948	0.592 ± 0.294
HOD-86	20	17	0.984	0.588 ± 0.274
APP-86	30	20	0.940	0.590 ± 0.303
APP-87	37	19	0.947	0.584 ± 0.333
RIV-86	24	15	0.953	0.552 ± 0.306
RIV-87	45	18	0.931	0.443 ± 0.254
SAR-86	87	28	0.953	0.558 ± 0.288
SAR-87	24	17	0.953	0.689 ± 0.295
1986	392	74	0.952	0.576 ± 0.304
1987	301	67	0.949	0.574 ± 0.310
Gulf	693	99	0.951	0.575 ± 0.308
Atlantic	174	43	0.904	0.560 ± 0.351

individuals from the Atlantic. Mean intrapopulational nucleotide sequence diversities among localities in the Gulf ranged from 0.443 to 0.704, with values for the two year-classes being essentially identical. Intrapopulational diversity values among localities from the Atlantic were comparable to those among localities from the Gulf. All estimated intrapopulational nucleotide-sequence diversity values, representing geographic localities, year-classes, and regions (i.e., Gulf and Atlantic) were within one standard deviation, indicating that levels of mtDNA variation (as measured by nucleotide-sequence diversity) are essentially identical throughout the geographic area surveyed.

Results of V-tests (using arcsine square-root-transformed haplotype frequencies), bootstrap analyses (after Roff and Bentzen 1989), and G-tests for spatial heterogeneity in haplotype frequencies are shown in Table 3. Tests were carried out (i) among Gulf localities within each year-class, (ii) among Gulf localities with year-classes at each locality pooled, (iii) among Gulf and Atlantic localities with year-classes at each locality pooled, and (iv) between pooled samples (1986 and 1987 year-classes) from the Gulf versus those from the Atlantic. As in Gold and Richardson (1991), all V-tests were carried out only on haplotypes that were found in four or more individuals in any spatial comparison grouping. Following corrections for multiple tests, no significant V-tests were found in comparisons of Gulf localities by year-class or with the 1986 and 1987 year-classes pooled, one significant V-test (for Haplotype 9) was found in the comparison of Gulf and Atlantic localities with the 1986 and 1987 year-classes pooled, and four significant V-tests (for Haplotypes 9, 12, 14, 19) were found in the comparison of pooled samples from the Gulf versus those from the Atlantic. Bootstrap probabilities indicated significant heterogeneity ($P < 0.05$) among Gulf localities in the 1986 year-class, among Gulf localities with the 1986 and 1987 year-classes pooled, and in both comparisons of Gulf red drum versus those in the Atlantic. Significant G-tests ($P < 0.05$) were found among Gulf localities in the 1987 year-class, among Gulf localities with the 1986 and 1987 year-classes pooled, and in both comparisons of Gulf red drum versus those in the Atlantic.

The haplotype-frequency heterogeneity within the Gulf detected in bootstrap analyses and G-tests appears to be due to Haplotypes 8 (1986 year-class), 11 (1987 year-class), and 10 and 31 (1986 and 1987 year-classes pooled). This follows from significant V-tests found for these four haplotypes in their respective groupings prior to correction for multiple tests. No consistent geographic trends (e.g. east to west clines) in frequency were apparent for three of the haplotypes (8, 10, and 11), suggesting that the heterogeneity detected was due simply to random frequency variations at different localities. A geographic trend across the Gulf was observed for Haplotype 31: frequencies of this haplotype were low in localities from the eastern Gulf and elevated in localities from the western Gulf. The clinal variation observed, however, may be anomalous given the low frequency (1.7%) of Haplotype 31 among all individuals sampled.

Heterogeneity of haplotype frequencies between pooled samples from the Gulf and those from the Atlantic appears to be real. The frequencies of Haplotype 9 at each Gulf and Atlantic locality (1986 and 1987 year-classes pooled) are shown in Fig. 2. With the exception of the sample from North Inlet, South Carolina (GTN), where only 18 individuals were assayed, frequencies of Haplotype 9 in Atlantic localities were approximately two-fold greater than those in Gulf localities. Haplotype 9 was the second most frequent haplotype found in the study, occurring in 97 (11.2%) of the 869 individuals surveyed. The remaining three haplotypes (12, 14, and 19) found to be significantly heterogeneous in V-tests (corrected for multiple tests) between pooled samples from the Gulf and pooled samples from the Atlantic, occurred in low frequency (i.e., 0.5 to 0.7%). All three haplotypes were found only in localities from the Atlantic. The low incidence of these three haplotypes suggests that the observed heterogeneity could be due to sampling error or to the possibility that full siblings (which would have identical mtDNA molecules) might have been accidentally sampled. Alternatively, ten significant V-tests were found in the comparison of pooled Gulf versus pooled Atlantic samples prior to correction for multiple tests.

Results of V-tests, bootstrap analyses, and G-tests for temporal heterogeneity in haplotype frequencies are shown in Table 4. Tests were carried out (i) between samples from the 1986 and 1987 year-classes at each locality

Table 3. *Sciaenops ocellatus.* Results of tests for spatial heterogeneity in mtDNA haplotype frequencies among localities from Gulf of Mexico and Atlantic Ocean. F_{ST}: measure of variance in mtDNA haplotype frequencies; $N_e m_f$: effective number of female migrants per generation

Test group	No. of localities	No. of haplotypes tested	No. of significant "V" tests	P[a]	Results of G-tests	F_{ST}	$N_e m_f$
Gulf of Mexico							
1986 year-class	11	24	1[b]	0.032	$P>0.05$	−0.002	>10
1987 year-class	10	20	1[b]	0.408	$P\simeq0.01$	0.008	>10
1986+1987 year-classes	11	31	2[b]	0.006	$P\simeq0.01$	0.002	>10
Gulf vs Atlantic							
1986+1987 year-classes	16	39	1	0.000	$P\simeq0.02$	0.007	>10
Pooled	2	41	10[c]	0.000	$P<0.001$	0.022	>10

[a] P: probability based on bootstrap analysis (after Roff and Bentzen 1989)
[b] Non-significant when corrected for multiple tests
[c] Six of 10 haplotypes non-significant when corrected for multiple tests

Table 4. *Sciaenops ocellatus.* Results of tests for temporal heterogeneity in mtDNA haplotype frequencies among samples from Gulf of Mexico. Locality acronyms as in Table 1

Test group	No. of samples	No. of haplotypes tested	No. of significant "V" tests	P[a]	Results of G-tests	F_{ST}	$N_e m_f$
1986 vs 1987 year-classes (by locality)							
LLM	2	2	0	0.836	$P>0.05$	−0.015	>10
PAR	2	2	0	0.583	$P>0.05$	−0.013	>10
PCV	2	0	–	0.319	–	0.003	>10
GVB	2	5	0	0.759	$P>0.05$	0.006	>10
SAB	2	4	0	0.686	$P>0.05$	0.002	>10
GIL	2	8	0	0.193	$P>0.05$	0.005	>10
OSP	2	10	1[b]	0.130	$P\simeq0.03$	0.008	>10
APP	2	6	0	0.618	$P>0.05$	0.002	>10
RIV	2	7	1[b]	0.332	$P>0.05$	−0.002	>10
SAR	2	11	1[b]	0.051	$P\simeq0.04$	0.009	>10
1986 vs 1987 year-classes (pooled)	2	33	1[b]	0.817	$P>0.05$	−0.001	>10

[a] P: probability based on bootstrap analysis (after Roff and Bentzen 1989)
[b] Non-significant when corrected for multiple tests

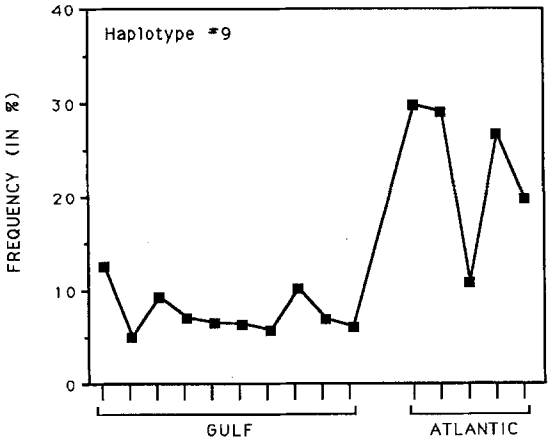

Fig. 2. *Sciaenops ocellatus.* Frequencies (%) of Haplotype 9 in 1986 and 1987 year-classes (pooled data) at 15 localities in Gulf of Mexico and Atlantic Ocean. Ten localities in Gulf (left to right on abscissa) are LLM, PAR, PCV, GVB, SAB, GIL, OSP, APP, RIV, and SAR. Five localities in Atlantic (left to right on abscissa) are HIH, CHS, GTN, BTH, and MNC. Acronyms for localities are given in Table 1

in the Gulf, and (ii) between 1986 and 1987 year-classes in the Gulf pooled over all localities. As before, all tests were carried out only on haplotypes that were found in four or more individuals in any temporal comparison grouping. Following corrections for multiple tests, no significant V-tests were found between the 1986 and 1987 year-classes at individual Gulf localities or between year-classes with localities pooled. Bootstrap probabilities were non-significant ($P>0.05$) for all comparisons, and significant G-tests were found only between the 1986 and 1987 year-classes at Sarasota Bay, Florida (SAR) and Biloxi Bay, Mississippi (OSP). In both cases, probability values were not far below the 5% level. These results indicate the absence of temporal genetic subdivision within the Gulf. In the Atlantic, no heterogeneity in haplotype frequencies was detected between samples from the 1986 and 1987 year-classes from Charleston Bay, South Carolina (CHS; data not shown).

Estimates of F_{ST} (a measure of the variance in mtDNA haplotype frequencies) and $N_e m_f$ (the effective number of female migrants per generation) are also shown in

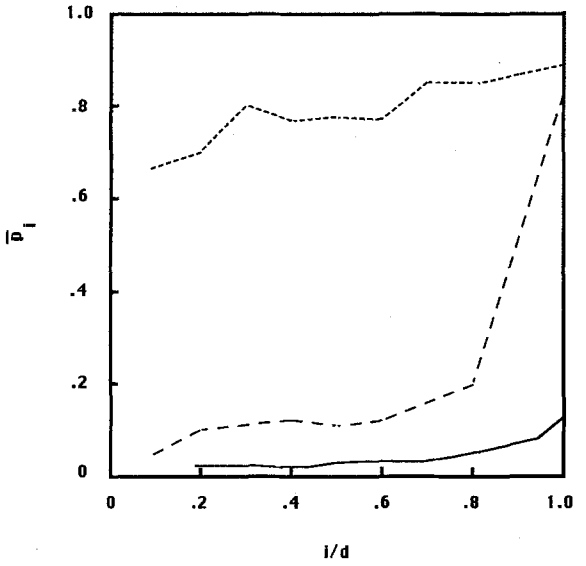

Fig. 3. *Sciaenops ocellatus.* Qualitative profile of gene flow among red drum (continuous line). Values obtained by plotting conditional-average allele (haplotype) frequencies (p_i) against occupancy rate (i/d) (see Slatkin 1981 for further details). Also shown are theoretical profiles (from Slatkin 1981) when $N_e m$ (effective number of migrants per generation) = 1.250 (dashed line) and 0.025 (dotted line). Theoretical profiles are based on (diploid) nuclear genes

Table 5. *Sciaenops ocellatus.* Results of tests of geographic subdivision in mtDNA haplotype frequencies in Gulf of Mexico. ECW: east (SAR, RIV, APP), central (OSP, HOD, GIL), west (SAB, GVB, PCV, PAR, LLM); RG 1–10: random groupings of three, three, and five localities (see "Results", Paragraph 9)

Test group	No. of haplotypes tested	No. of significant "V"-tests[a]	P[b]	Results of G-tests
ECW	33	8	<0.001	$P<0.001$
RG 1	33	3	0.252	$P>0.050$
RG 2	33	1	0.159	$P>0.050$
RG 3	33	6	0.001	$P<0.001$
RG 4	33	1	0.269	$P\simeq0.035$
RG 5	33	5	0.188	$P\simeq0.002$
RG 6	33	2	0.014	$P\simeq0.003$
RG 7	33	4	<0.001	$P<0.001$
RG 8	33	3	0.021	$P\simeq0.004$
RG 9	33	1	0.838	$P>0.050$
RG 10	33	3	0.362	$P\simeq0.009$

[a] All were non-significant when corrected for multiple tests
[b] P: probability based on bootstrap analysis (after Roff and Bentzen 1989)

Tables 3 and 4. For spatial groupings (Table 3), F_{ST} values ranged from −0.002 to 0.022; for temporal groupings (Table 4), F_{ST} values ranged from −0.015 to 0.009. $N_e m_f$ values, derived from F_{ST} values using the island model of Wright (1943), were >10 for all spatial and temporal comparisons (Tables 3 and 4). The estimates of F_{ST} and $N_e m_f$ indicate little genetic subdivision, and the occurrence of significant gene flow among spatial and temporal comparison groups, as shown in Tables 3 and 4.

Slatkin's (1981, 1985) qualitative and quantitative methods were used to estimate gene flow among all geographic localities. The qualitative method (Fig. 3) revealed a curve typical of species with high gene flow. The quantitative method employed 51 "private" alleles (haplotypes) among 11 Gulf localities and 63 "private" alleles (haplotypes) among 16 Gulf and Atlantic localities. Estimated $N_e m_f$ values were 40.50 and 24.07, respectively, for the Gulf and the Gulf and Atlantic. Both $N_e m_f$ values are considerably greater than 1, indicating high levels of gene flow among all localities.

To explore the possibility of regional subdivision within the Gulf, samples from both the 1986 and 1987 year-classes were pooled by locality and the localities pooled into three regions. The regions were: (i) eastern Gulf [samples from Sarasota Bay, Florida (SAR), Riviera Bay, Florida (RIV), and Apalachicola Bay, Florida (APP)]; (ii) central Gulf [samples from Biloxi Bay, Mississippi (OSP), Black Bay, Louisiana (HOD), and Grand Isle, Louisiana (GIL)]; and (iii) western Gulf [samples from Sabine Pass, Texas (SAB), Galveston Bay, Texas (GVB), Pass Cavallo, Texas (PCV), Aransas Pass, Texas (PAR), and Lower Laguna Madre, Texas (LLM)]. Haplotype frequencies at the three regions were tested for heterogeneity using V-tests, bootstrap analyses, and G-tests. As before, V-tests were carried out only on haplotypes found in four or more individuals. Eight haplotypes were found to differ significantly ($P<0.05$) in V-tests, although none were significant when corrections were made for multiple tests. The bootstrap analysis and G-test yielded probabilities <0.001, indicating significant heterogeneity among the three regions in haplotype frequencies. Since nearly all of the haplotypes found to be significant in the (uncorrected) V-tests were low-frequency haplotypes in the Gulf, the possibility that significant heterogeneity could be detected by chance alone was tested by bootstrapping the data set. The 11 geographic localities were randomly assigned to 10 random groupings of 3, 3, and 5 localities, thus mimicking the original groupings in terms of the number of geographic localities per grouping. Haplotype frequencies among the 10 random groupings (designated RG 1-10) were tested for heterogeneity using V-tests, bootstrap analysis, and G-tests. The results are shown in Table 5, along with the results for the original eastern–central–western Gulf grouping. In V-tests, from 1 to 6 haplotypes were found to differ significantly ($P<0.05$) in frequency among the 10 random groupings. None of the V-tests were significant when corrections were made for multiple tests. Significant heterogeneity ($P<0.05$) was found in 4 of the 10 random groupings using bootstrap analysis, and in 7 of the 10 random groupings using G-tests. These results indicate that the heterogeneity detected among the original eastern–central–western grouping could be due to chance alone rather than geographic subdivision. It is worth noting, however, that the number of haplotypes whose frequencies differed significantly in uncorrected V-tests was greater in the original grouping than in any of the 10 random groupings. Moreover, the probability values observed for the original grouping in both the bootstrap analyses and G-tests were lower than those found in

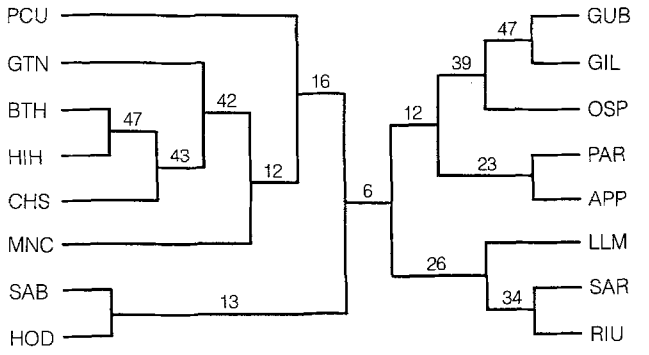

Fig. 4. *Sciaenops ocellatus.* Strict (unrooted) consensus tree produced by parsimony analysis of a binary coded (presence/absence) restriction-site matrix. Numbers along branches indicate proportion of times (from 100 replicates) that a branch (clade) was distinguished in bootstrap analysis. Branch lengths are not accurate representations of number of character-state changes between operational units. Operational units are all Gulf and Atlantic localities (1986 and 1987 year-classes pooled). Acronyms for localities are given in Table 1

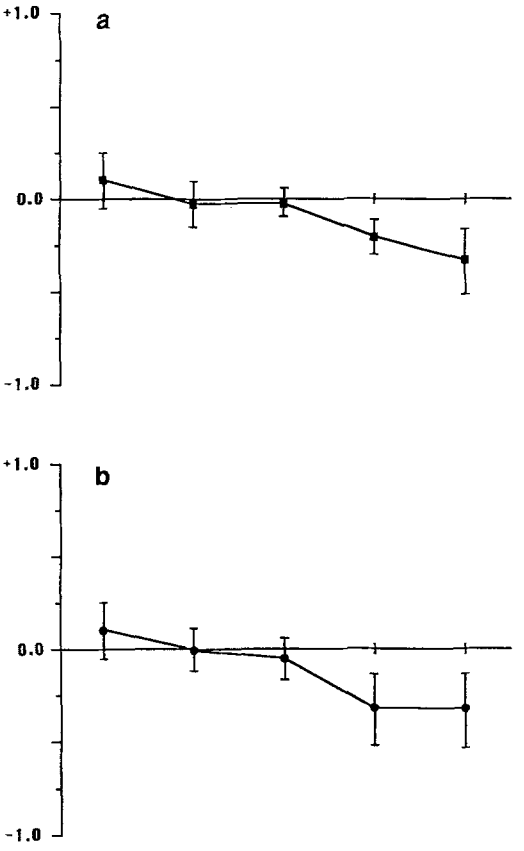

Fig. 5. *Sciaenops ocellatus.* Correlograms based on frequencies of haplotypes found in 10 or more individuals among 11 geographic localities from the Gulf. Abscissas: Distance Classes 1–5 (left to right); ordinates: mean autocorrelation coefficients (Moran's *I*-values) for each distance class. Bars about each mean value represent two standard errors on either side of a mean. (a) Equal frequencies/distance class; (b) equal distances between distance classes

any of the 10 random groupings. This suggests that there may be a small, but noticeable regional effect on haplotype frequencies.

Estimates of nucleotide sequence divergence were generated (i) among all Gulf and Atlantic localities by year-class, and (ii) among all Gulf and Atlantic localities with year-classes pooled. (The matrices of nucleotide-sequence divergence are not shown, but may be obtained upon request from the first author.) Cluster analysis using the UPGMA algorithm was used to summarize the nucleotide-sequence divergence matrices. In both phenograms, standard errors of the most distant nodes were greater than the distance between the first and last nodes, effectively collapsing all of the nodes and indicating that all 16 geographic samples of red drum (by year-class or with year-classes combined) are not strongly differentiated genetically.

Parsimony analysis of sample restriction-site presence/absence matrices representing (i) all haplotypes, and (ii) all Gulf and Atlantic localities with year-classes pooled were carried out using PAUP. The strict consensus "gene tree" produced from the haplotype matrix essentially revealed a single large polytomy. The strict consensus tree produced from the matrix of Gulf and Atlantic localities is shown in Fig. 4. Results from bootstrapping (100 replicates) indicated very little support for phyletic cohesion of geographically proximate localities from the Gulf. Weak support (42 to 47% bootstrapping) for phyletic cohesion of 4 of 5 Atlantic localities [Pamlico River, North Carolina (BTH), North Inlet, South Carolina (GTN), Charleston Bay, South Carolina (CHS), and Calibogue Sound, South Carolina (HIH)] paralleled results of heterogeneity testing, whereby samples from the Atlantic were found to be differentiated genetically from samples from the Gulf.

Spatial autocorrelation analyses were used to determine whether haplotype frequencies at any Gulf locality were independent of haplotype frequencies at neighboring localities. SAAP runs using haplotypes found in four or more individuals generated 165 Moran's *I*-values (33 haplotypes × 5 distance classes). When equal numbers of pairwise comparisons were used, 20 significant ($P < 0.05$) values were obtained; 28 significant values were obtained when equal geographic distances between distance classes were used. The distribution of significant values was nonrandom: positive values were found primarily in the first or second distance classes, whereas negative values were found primarily in the last distance class. Nearly identical results were obtained in SAAP runs using only haplotypes found in 10 or more individuals. Graphical representations of these results are shown in Fig. 5 where the mean (± 2 SE) Moran's *I*-values, averaged over all haplotypes occurring in 10 or more individuals, are plotted by distance class. In both SAAP runs [i.e., using equal frequencies (Fig. 5a) or using equal distances (Fig. 5b)], mean Moran's *I*-values are positive in the first distance class, near zero in the second and third distance classes, and negative in the last two distance classes. These results indicate a weak autocorrelation among haplotype frequencies as a function of distance between pairs of localities; i.e., red drum in geographically proximate or neighboring localities are more similar genetically than are red drum in more geographically distant localities.

Table 6. Estimates of mtDNA variability and of evolutionary effective female population sizes among species of marine fishes. Parentheses indicate number of mtRNA restriction sites or fragments surveyed. $N_{f(e)}$: evolutionary effective-female population size, estimated after Avise et al. (1988). Assumed generation times for each species are given in text

Species	No. of individuals surveyed	No. of different mtDNA haplotypes	Nucleotide sequence diversity (%)[a]	Evolutionary effective female population size [$N_{f(e)}$]	Source
Atlantic menhaden	17	17 (55)	3.19	800 000	Avise (1992)
Atlantic herring	69	26 (77)	1.09	273 000	Kornfield and Bogdanowicz (1987)
Gulf menhaden	16	16 (55)	0.99	250 000	Avise (1992)
Gulf red drum	695	99 (104)	0.57	95 000	Present study
Atlantic red drum	174	43 (104)	0.56	93 000	Present study
Spotted seatrout	70	14 (50)	0.30	75 000	Furman and Gold (unpublished data)
Greater amberjack	59	23 (102)	0.34	57 000	Richardson and Gold (1993)
Black drum	250	19 (67)	0.17	17 000	Furman and Gold (unpublished data)
Red grouper	51	12 (92)	0.08	10 000	Richardson and Gold (1993)
Black sea bass[b]	19	3 (61)	0.03	5 000	Avise (1992)
Black sea bass[c]	10	2 (61)	0.03	5 000	Avise (1992)

[a] Intrapopulational nucleotide sequence diversity values
[b] Atlantic subspecies (*Centropristis striata striata*)
[c] Gulf subspecies (*Centropristis striata melana*)

Effective female population sizes

Avise et al. (1988) presented theoretical models whereby estimates of evolutionary effective female-population size ($N_{f(e)}$ values) of a species (or a distinct subpopulation or stock of a species) can be generated from estimates of mtDNA intrapopulational nucleotide-sequence diversity. Estimates of $N_{f(e)}$ for red drum from the Gulf and Atlantic are shown in Table 6 along with comparable estimates for several other marine fishes of commercial or recreational importance. Data for species other than red drum were taken either from the published literature or from work in our laboratory. Assumed generation lengths were 2 yr (Atlantic and Gulf menhaden, Atlantic herring, and spotted seatrout), 3 yr (red drum, black sea bass, and greater amberjack), and 5 yr (black drum and red grouper), and were based either on Avise (1992) or personal communications from L. Bullock (red grouper), I. Kornfield (Atlantic herring), and C. Wilson (red drum, spotted seatrout, greater amberjack, black drum). Estimates of $N_{f(e)}$ are highest for the clupeid species (menhaden and herring), intermediate for red drum, spotted seatrout, and greater amberjack, and lowest for black drum, red grouper, and black sea bass. $N_{f(e)}$ values for red drum are the highest of all the non-clupeid species.

Discussion

Sciaenops ocellatus in the Gulf appear to be weakly differentiated genetically from red drum in the Atlantic. Heterogeneity tests revealed significant frequency differences in at least four mtDNA haplotypes, one of which (Haplotype 9) is fairly common in both the Gulf and Atlantic. The degree of genetic differentiation between Gulf and Atlantic red drum, however, is slight. Cluster-analysis of mtDNA genetic distances indicated that red drum from individual Gulf and Atlantic localities were not distinguishable from one another, and parsimony analysis revealed only weak support for phyletic cohesion of samples from the Atlantic. In addition, Slatkin's (1981) qualitative method and estimates of the effective number of migrants per generation using Wright's (1943) island model and Slatkin's (1985) quantitative method, indicated that gene flow between red drum in the Gulf and Atlantic is relatively high. The observation of genetic differentiation and high gene flow is not unusual, since (i) significant genetic divergence can occur even if substantial gene exchange exists, and (ii) small values of F can be associated with a considerable amount of genetic differentiation between or among subpopulations (Wright 1969).

In nearly all respects, mtDNA differentiation between Gulf and Atlantic red drum is fully concordant with differentiation in nuclear genes. Gold et al. (1993) surveyed nine polymorphic (allozyme) loci among most of the red drum examined for mtDNA, and found weak but significant differentiation between Gulf and Atlantic red drum in at least two nuclear genes. Estimated levels of gene flow were high and little geographic cohesion was evident from cluster analysis of genetic distances. Taken together, the genetic data indicate that red drum from the Gulf and Atlantic are weakly subdivided, and for management purposes should be considered as separate assemblages (stocks). The biological reasons for subdivision between Gulf and Atlantic red drum are unknown. Historical or recent interactions between red drum dispersal and impediments to gene flow appear to be the most likely explanations (Gold and Richardson 1991), and may include one of more of the following: (i) offshore currents are not conducive to movement of red drum between the Gulf and Atlantic; (ii) absence of suitable nearshore or other habitats for red drum along the southeastern (Atlantic) coast of Florida; and/or (iii) differences in biogeographic provinces separating Gulf from Atlantic marine fauna.

The results of the present study indicate the effective absence of spatial and temporal genetic differentiation among red drum from the Gulf; i.e., red drum in the Gulf

appear to comprise a single, randomly mating subpopulation. This conclusion is drawn from several lines of evidence, including (i) spatial (among localities) and temporal (between year classes) heterogeneity tests of mtDNA haplotype frequencies, (ii) phenetic (cluster) and parsimony analysis of Gulf localities, and (iii) estimates of the effective number of migrant individuals per generation among localities. Nearly identical results (i.e., the absence of population structuring among Gulf red drum) were found by Gold et al. (1993) in their study of allozyme differentiation.

Spatial autocorrelation analysis of the distribution of mtDNA haplotypes over all Gulf localities indicated that geographically proximate or neighboring localities were more similar genetically than were more geographically distant localities. The observed pattern of autocorrelation is consistent with an "isolation-by-distance" model, whereby migration of individuals within the Gulf is inversely related to geographic distance from the estuary or bay of natal origin. Given the known life-history of red drum (Overstreet 1983, Matlock 1984, 1987, Mercer 1984, Reagan 1985, Lyczkowski-Schultz et al. 1988, Murphy and Taylor 1990), such migration could occur: (i) at the egg, larval, or juvenile stages following offshore spawning; (ii) at the sub-adult stage (Ages 1 to 3); and/or (iii) at the adult stage following movement into deeper waters prior to sexual maturity and spawning.

Spatial autocorrelation analysis of nuclear-gene (allozyme) variation among Gulf red drum did not reveal a significant pattern of autocorrelation (Gold et al. 1993). The finding of autocorrelation in the mtDNA data, but not in the nuclear-gene data, is not surprising given (i) the effect, if it is real, is only slight, and (ii) the increased sensitivity of mtDNA (relative to nuclear genes) in measuring the genetic impact of population substructuring and gene flow (Birky et al. 1983, Templeton 1987). From a "nearshore" management perspective, the implication of an "isolation-by-distance" effect is that management strategies (e.g. fishing regulations) for a given estuary or bay would be likely to impact neighboring estuaries or bays more than geographically distant ones. From an "offshore" management perspective, however, the absence of strong local or regional genetic differentiation, coincident with sufficient levels of migration to offset genetic diversification, indicates that adult red drum in the Gulf should be considered (and managed) as a single unit assemblage (stock).

Estimates of mtDNA nucleon and nucleotide-sequence diversities indicated that genetic variation in red drum is equivalent to, or higher than that in most other marine fish species surveyed to date (Gold and Richardson 1991). Importantly, high levels of mtDNA variation were found at all geographic localities (by year-class and with year-classes pooled) in both the Gulf and Atlantic, indicating that red drum have at least normal levels of genetic variation throughout the geographic area surveyed. Similar results, i.e., levels of genetic variation in red drum similar to those in many other vertebrates, including several marine fishes, were also found in the survey of nuclear genes (Gold et al. 1993). These data collectively indicate that the perceived decline in red drum abundance, at least in the Gulf, has not affected either the genetic variability base or the long-term adaptive potential of red drum throughout the Gulf.

Estimates of evolutionary-effective female-population sizes yielded $N_{f(e)}$ values of slightly less than 100 000 red drum females in both the Gulf and Atlantic. As discussed by Avise et al. (1988) and Avise (1992), $N_{f(e)}$ estimates are thought to reflect evolutionary or long-term effective sizes of female populations and appear to significantly underestimate present-day female population (census) sizes. Part of the reason for this may be rates of mtDNA-sequence evolution that are lower than the conventionally-accepted rate (Wilson et al. 1985) used to generate $N_{f(e)}$ estimates. Avise et al. (1988), however, have shown that this would require a molecular deceleration of 200- to 900-fold, a possibility that seems unlikely given the number of distinct mtDNA haplotypes found in species such as the red drum. A more likely possibility would be differences in the number of females through which extant mtDNA lineages have been transmitted (Avise et al. 1988, Avise 1992). In theory, the latter could be affected by historical demography, overlapping generations, or selective fitness differentials among mtDNA haplotypes (Avise 1992).

Avise et al. (1988) and Avise (1992) have shown that a positive correlation exists between $N_{f(e)}$ and N_f values, where the latter represents the present-day size of the female population, and that estimates of $N_{f(e)}$ for several marine fish species are typically two orders of magnitude lower than estimates of N_f. Assuming similar conditions pertain to red drum, the $N_{f(e)}$ value of 95 000 for red drum from the Gulf suggests a female population size in the Gulf on the order of 9.5 million. Assuming a 1:1 sex ratio among adult red drum, the total size of the red drum population in the Gulf could be on the order of 19 million individuals. Nichols (1988), using mark/recapture methods, estimated adult red drum (female and male) density in the northern Gulf to be approximately 7 million individuals. Given the uncertain relationship between $N_{f(e)}$ and N_f values, Nichol's estimate appears reasonable and could be considered as a minimum estimate of the adult red drum population in the northern Gulf.

The estimated $N_{f(e)}$ values (and the nucleotide-sequence divergence values from which $N_{f(e)}$ values are derived) may be useful as indices of long-term adaptive potential. The latter is based on the concepts that levels of genetic variability may affect probabilities of populational survival and fitness (Soulé 1980, Frankel and Soulé 1981), and that genetic characters may be used to assess levels of genome-wide variability (Wildt et al. 1987, Quattro and Vrijenhoek 1989). Levels of extant mtDNA variability appear to vary considerably among marine fish species, and levels of mtDNA variability in red drum are considerably higher than in most other, non-clupeid species thus far surveyed. This further indicates that the perceived decline in red drum abundance (Matlock 1984, Reagan 1985) has not affected either the genetic variability base or the long-term adaptive potential of this species.

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