Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and the western Atlantic Ocean*

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Abstract—Variation in restriction sites of mitochondrial (mt)DNA was examined from 444 greater amberjack (*Seriola dumerili*) sampled from 11 offshore localities in the northern Gulf of Mexico (Gulf) and along the U.S. southeast Atlantic coast (Atlantic). A total of 49 mtDNA haplotypes (genotypes) were detected. Percent nucleotide sequence divergence among the haplotypes ranged from 0.156 to 2.623 (mean ±SE=0.980 ±0.015). Nucleon diversity within samples ranged from 0.845 to 0.906, and intrapopulational mtDNA diversities ranged (mean ±SD) from 0.483 ±0.370 to 0.619 ±0.419. The latter did not differ significantly from one another. Homogeneity tests of mtDNA haplotype frequencies, F_{ST} values, analysis of molecular variance (AMOVA), and comparisons of pairwise Φ_{ST} distances were consistent with the hypothesis that (at least) two subpopulations (stocks) of greater amberjack exist in U.S. waters: one in the northern Gulf and one along the U.S. Atlantic coast. The latter subpopulation includes individuals from the Florida Keys. There was no evidence of phylogeographic structure among mtDNA haplotypes or among sample localities, suggesting either that restricted gene flow between the subpopulations is fairly recent or that gene flow between the two is relatively infrequent. No significant spatial autocorrelations of mtDNA haplotypes was found among samples of greater amberjack from the Gulf, indicating continuous gene flow across the northern Gulf. Long-term effective (female) population sizes of both subpopulations were estimated to be in the range of 90,000–95,000 individuals. The estimates were commensurate with estimates in other, economically important marine fish. Based on suggested differences in mtDNA evolutionary rates between homeothermic and poikilothermic vertebrates, the effective (female) population sizes of both stocks of greater amberjack could be in the range of 500,000 to 1,000,000 individuals.

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Greater amberjack, *Seriola dumerili*, constitute an increasingly important component of the U.S. Gulf and Atlantic commercial and recreational fisheries. Commercial landings in the Atlantic rose over twentyfold between 1981 and 1991, from under 100,000 pounds annually to nearly two million pounds (Cummings-Parrack[1]). Commercial landings in the Gulf rose similarly, reaching a peak of nearly 2.5 million pounds in 1988 that was then followed by a >50% decline in 1990 and a further decline in 1991 (McClellan and Cummings[2]). An increase in commercial landings in 1993 was followed by declines in both 1994 and 1995 (McClellan and Cummings[2]). The commercial interest in greater amberjack appears to be driven by: 1) consumer acceptance of greater amberjack as an edible fish; 2) replacement of red drum in the blackened fish market; and 3) effort displacement of king mackerel and reef-fish fishermen, especially off the coasts of Florida (Cummings-Parrack[1]). Recreational landings are smaller in both the Gulf and Atlantic and appear to have declined in recent years (Cummings-Parrack[1]; McClellan and Cummings[2]). Problems that confound landing statistics and fishery analysis of the greater amberjack resource include species misidentification and the possibility that reported landings may be small in proportion to total removals (Cummings-Parrack[1]). Current management of the greater amberjack resource in U.S. waters is based on a two-stock (management unit) model. One stock resides along the U.S. southeastern coast and includes all fishing areas north of Cape Hatteras, NC (35°00'N) southward to the Dry Tortugas and the Florida Keys (24°35'N); the other includes fishing grounds in the Gulf of Mexico off the Dry Tortugas and Florida Keys north and then westward to the U. S. Mexico border (26°00'N)

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[1] Cummings-Parrack, N. 1993. The exploitation status of the Atlantic amberjack fisheries through 1991. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-92/93-30, Miami, FL, 98 p.

[2] McClellan, D. B., and N. J. Cummings. 1996. Stock assessment of Gulf of Mexico greater amberjack through 1995. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-96/97-03, Miami, FL, 69 p.

(Cummings and McClellan[3]). Movement patterns inferred from mark-and-release experiments carried out between 1959 and 1994 (Cummings and McClellan[3]) are consistent with the two-stock hypothesis. In brief, over 1400 recaptures from approximately 14,000 releases revealed cyclical tag-return patterns that suggested resident stocks or subpopulations of greater amberjack along both the eastern coast of Florida and the northern Gulf. Exchange rates between the two stocks was estimated as approximately 1.5% by Cummings and McClellan[3] although, as noted by these authors, the rate estimates were not adjusted for fishing pressure or for potential biases due to mortality, tag shedding, lack of reporting, and fishing effort. It also was clear from a few tag returns that greater amberjack can migrate considerable distances, e.g. from near Charleston, South Carolina, to Texas or from northwest Florida to Virginia (Cummings and McClellan[3]).

Biological information on greater amberjack is limited to studies reported in Berry and Burch (1978), Shipp (1986), Manooch (1988), and GMFMC (1989). Thompson et al.[4] presented data on greater amberjack age, growth, and reproduction, and Cummings-Parrack[1] and McClellan and Cummings[2] summarized most of the available information on landings and other fishery statistics. Direct or indirect information on genetic stock structure is even more limited. Johnson[5] carried out a pilot study of nuclear-gene (allozyme) variation among 225 greater amberjack sampled from the Atlantic (n=60), eastern Gulf (n=84), and western Gulf (n=81). Of 72 putative loci examined, only one polymorphic (and noninformative) system was found. On the surface, these data do not support the concept of separate stocks. However, genetic homogeneity (*sensu stricto*) does not unequivocally establish the existence of a single breeding population (stock), but rather is simply consistent with the hypothesis that samples are drawn from a population with the same parametric allele frequencies. In addition, the almost total absence of variation effectively precluded rigorous testing of the null hypothesis (i.e. the interpretation of genetic homogeneity among samples is potentially compromised by virtue of the absence of significantly variable nuclear-gene loci).

Alternatively, the apparent absence of genetic variation raises considerable concern about the effective size of greater amberjack populations. Compared with other marine finfish (Smith and Fujio, 1980; Waples, 1987; Bohlmeyer and Gold, 1991), levels of nuclear-gene variation in greater amberjack (as reported by Johnson[5]) are low. Richardson and Gold (1993) examined mitochondrial (mt)DNA variation among 59 greater amberjack sampled primarily from the west coast of Florida. Levels of mtDNA variation in greater amberjack were low in comparison with red drum and several clupeid species (e.g. Atlantic menhaden), but higher than those found in black drum, red snapper, and red grouper (Camper et al., 1993; Gold et al., 1993; Richardson and Gold, 1993). Estimates of long-term, effective female population size (computed directly from levels of mtDNA variation) paralleled levels of mtDNA variation, suggesting that effective (female) population sizes of Gulf greater amberjack were not atypically low.

Concerns regarding greater amberjack fisheries in the Gulf and Atlantic include the following: 1) presumed decreases in average individual size in both Gulf and Atlantic fisheries; 2) apparent declines in size of the presumed Atlantic stock; 3) a trend of declining yield in both commercial and headboat fisheries in the Gulf; and 4) apparent highly erratic recruitment where success of individual year classes is quite variable (Cummings-Parrack[1]; Cummings and McClellan[3]). These concerns have intensified as the economic importance of greater amberjack has grown (GMFMC, 1989; Cummings and McClellan[3]). In this study, we employed variation in restriction sites in mitochondrial (mt)DNA of greater amberjack to determine if significant population structure (separate genetic stocks) occurs in U.S. waters, i.e. in the northern Gulf of Mexico and along the U.S. southeastern Atlantic coast. The rationale for this study is the need for accurate geographic definition when conducting stock assessments (Hilborn, 1985; Sinclair et al., 1985), in this case for greater amberjack in U.S. waters.

Materials and methods

Appropriate tissues (heart and white muscle) were obtained from a total of 444 greater amberjack sampled from 11 offshore localities in U.S. waters (Table 1; Fig. 1). With exception of a sample of seven individuals from near Gulfport, MS, sample sizes

[3] Cummings, N. J., and D. B. McClellan. 1996. Movement patterns and stock interchange of greater amberjack *Seriola dumerili*, in the southeastern U.S. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-95/96-14, Miami, FL, 24 p.

[4] Thompson, B. A., C. A. Wilson, J. H. Render, H. Beasley, and C. Cauthron. 1992. Age, growth, and reproductive biology of greater amberjack and cobia from Louisiana waters. Final Rep., Marfin Prog., U.S. Dep. Comm., Coop. Agreement NA90AA-H-MF722, 77 p.

[5] Johnson, A. G. 1990. Progress report: electrophoretic examination of greater amberjack (*Seriola dumerili*). Panama City Laboratory, SE Fish. Sci. Center, Natl. Mar. Fish. Serv., Panama City, FL, 34 p.

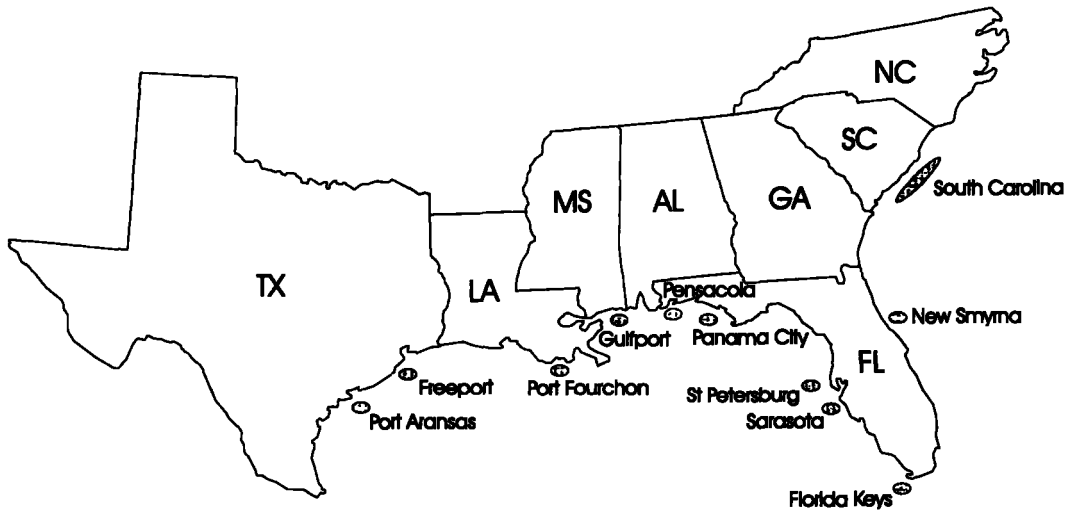

Figure 1

Dockside localities for greater amberjack, *Seriola dumerili*, in present study: NC = North Carolina; SC = South Carolina; GA = Georgia; FL = Florida; AL = Alabama; MS = Mississippi; LA = Louisiana; and TX = Texas.

ranged from 24 to 58 individuals. Individuals were sampled variously from charter boats, headboats, and commercial fishing boats, and from catches of recreational fishers at tournaments. Tissues were removed from each specimen, quickly frozen in liquid nitrogen, and transported to Texas A&M University where they were stored at –80°C in an ultracold freezer.

Methods (including DNA extraction, precipitation, and storage) used to assay restriction-enzyme fragment patterns of mtDNA molecules of individual fish followed those described in Gold and Richardson (1991). Sixteen, type-II restriction-endonuclease enzymes were used to digest 1.0–1.5 µg of DNA in 40 µL reactions according to manufacturer's specifications. Enzymes used were *Apa*LI, *Apa*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco*I, *Pst*I, *Pvu*II, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Stu*I, and *Xba*I. Methods of DNA digestion, agarose electrophoresis, transfer to nylon filters (after Southern, 1975), hybridization, and autoradiography also followed those in Gold and Richardson (1991). Hybridization employed a 12.5 kilobase (kb) fragment of greater amberjack mtDNA cloned into lambda bacteriophage (Richardson and Gold, 1993). Bacteriophage lambda DNA digested with restriction enzyme *Hin*dIII was employed as a molecular weight marker on each gel. MtDNA fragments produced by single digestion of greater amberjack mtDNA were sized by fitting migration distances to a least-squares regression of lambda DNA-*Hin*dIII fragment migration distances. Single digestion mtDNA-fragment patterns were used to generate composite digestion patterns (mtDNA haplotypes).

Table 1

Sample localities of greater amberjack (*Seriola dumerili*).

Locality[1]	Number of individuals
Port Aransas, Texas (PA)	58
Freeport, Texas (FP)	44
Port Fourchon, Louisiana (PF)	43
Gulfport, Mississippi (GP)	7
Pensacola, Florida (PN)[2]	24
Panama City, Florida (PC)	50
St. Petersburg, Florida (SP)	42
Sarasota, Florida (SR)[2]	32
Florida Keys (FK)	55
New Smyrna Beach, Florida (NS)	53
South Carolina (SC)	36
Total	444

[1] Localities represent dock sites where individuals sampled offshore were off-loaded. Individuals from the Florida Keys were off-loaded at Islamorada; individuals from South Carolina were off-loaded at several localities.
[2] Previously examined by Richardson and Gold (1993).

Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Genotypic (nucleon) diversity within sample localities was calculated following Nei and Tajima (1981) and was based on the total number of mtDNA haplotypes identified within a local-

ity. This value represents the probability that any two individuals drawn at random will differ in mtDNA haplotype. Intrapopulational nucleotide sequence diversity within sample localities also was estimated after Nei and Tajima (1981). This value represents the average nucleotide sequence difference between any two individuals drawn at random from a given sample or locality.

Homogeneity testing of mtDNA haplotype frequencies among sample localities was carried out by using 1) a randomization (bootstrap) procedure developed by Roff and Bentzen (1989), 2) a log-likelihood (G) test (Sokal and Rohlf, 1969), and 3) V tests that employed arcsine, square-root-transformed haplotype frequencies (DeSalle et al., 1987). Significance levels for multiple tests were adjusted after Rice (1989). F_{ST} values, a measure of the variance in mtDNA haplotype frequencies, were calculated after Weir and Cockerham (1984) by using algorithms described in Weir (1990). Significance of F_{ST} values was tested by the randomization procedure in version 1.4 of the analysis of molecular variance (AMOVA) program of Excoffier et al. (1992). The latter program (AMOVA) was used primarily to examine the distribution of variance in mtDNA haplotypes. AMOVA analysis generates estimates of (genetic) variance components and a set of hierarchical F-statistic analogs (Φ statistics) that are tested for significance through random permutation methods. The permutation approach avoids the parametric assumptions of normality and independence normally not met by molecular distance measures (Excoffier et al., 1992). Sample localities were nested into regional groupings, i.e. Gulf and Atlantic, that were input into AMOVA. In one AMOVA run, the sample from the Florida Keys was included with the Gulf group, whereas in a second run it was included in the Atlantic group.

Restriction-site presence and absence matrices for individual mtDNA haplotypes were constructed with the GENERATE program in REAP by inferring restriction site gains and losses for each enzyme. Maximum-parsimony analysis of restriction-site presence and absence matrices representing all haplotypes employed MULPARS and CONTREE options in version 3.1 of the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1991). Autapomorphic and symplesiomorphic characters were removed prior to PAUP runs. Nucleotide-sequence divergence values among sample localities (interpopulational divergence) were generated following Nei and Tajima (1981) and Nei and Miller (1990). MtDNA-based similarity among sample localities was assessed with the neighbor joining method (Saitou and Nei, 1987) and employed the NEIGHBOR program in Phylogenetic Inference Package (PHYLIP), version 3.4 of Felsenstein (1989).

The spatial distribution of mtDNA haplotypes was investigated by means of spatial autocorrelation analysis (SAAP; Wartenberg, 1989). This analysis determines whether haplotype frequencies at any sample locality are independent of haplotype frequencies at neighboring sample localities. Correlograms that plot autocorrelation coefficients (Moran's I values) as a function of geographic distance between pairs of localities were used to summarize patterns of geographic variation of haplotype frequencies. To minimize "noise" generated by low-frequency haplotypes, Moran's I values were calculated only for haplotypes occurring in eight or more individuals (10 haplotypes total). These included haplotypes 1–4, 6, 11, 13, 23, 37, and 48.

Results

Single digestions of mtDNA molecules from the 444 individuals surveyed produced variable fragment patterns for the 16 restriction enzymes. The majority of fragment patterns observed are given in Appendix Table A2 of Richardson and Gold (1993). New fragment patterns revealed in our study are as follows (fragment sizes in base pairs; asterisks represent fragments assumed to exist but not covered by the mtDNA probe): *Eco*RI, pattern C (8700, 5025, 3175*); *Hpa*I, pattern C (8800, 5550, 2550); *Nco*I, pattern C (11050, 5850); *Sst*I, pattern C (11700, 3450, 1750); *Sca*I, pattern C (7500, 3600, 3100, 2700) and pattern D (10000, 3600, 3300); *Sma*I, pattern D (10700, 4250, 1500, 450); *Spe*I pattern D (7900, 5800, 1200, 1200*, 800); *Ssp*I, pattern C (6600, 6200, 4100); and *Xba*I, pattern D (7300, 5000, 4600) and pattern E (7300, 4600, 2600, 2275, 125*). The mean genome size of all apparently complete digestion patterns was 16.9 ±0.2 kilobase pairs. No evidence of mtDNA size variation or heteroplasmy was observed. All fragment patterns for each restriction enzyme were consistent with the hypothesis of single nucleotide substitutions. A total of 72 unique restriction sites was inferred from the digestion patterns.

A total of 49 mtDNA haplotypes was identified among the 444 individuals surveyed (Table 2). Four haplotypes (8, 14, 16, and 18) are not listed in Table 2; these were listed in Richardson and Gold (1993) and were identified by three restriction enzymes (*Cla*I, *Nsi*I, and *Pvu*I) not employed in our study. Four haplotypes (1, 4, 6, and 13) were abundant, occurring in 77, 91, 54, and 70 individuals, respectively. Two haplotypes (2 and 3) occurred in 20 and 22 individuals, respectively, whereas the remainder occurred in 10 or fewer individuals. Twenty haplotypes were found in only one individual each. Estimates of

Table 2

Spatial distribution of mitochondrial (mt)DNA haplotypes of greater amberjack (*Seriola dumerili*). Letters (from left to right) are digestion patterns for *Apa*LI, *Apa*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco*I, *Pst*I, *Pvu*II, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Stu*I, and *Xba*I.

Haplotype no.	Composite mtDNA genotype	Samples										
		PA	FP	PF	GP	PN	PC	SP	SR	FK	NS	SC
1	AAAAAAAAAAAAAAAA	7	10	8	2	5	8	12	7	4	8	6
2	AAAABBAAAAAAAAAA	4	4	3	—	1	2	3	—	3	2	1
3	AAAAABAAAAAAAABA	3	2	3	—	3	1	1	—	4	4	1
4	AAAAABAAAAAAAAAA	15	9	10	1	5	11	4	10	10	9	7
5	AABBABAAAABAABAA	—	1	—	—	3	—	—	1	—	—	—
6	AAAAAAAAAAAAAABA	8	4	2	1	—	4	4	5	10	6	10
7	AAAAAAAAAABAAAA	—	—	—	—	—	—	—	1	—	—	—
9	BABAAAAAAACAAABA	—	—	—	1	—	—	—	1	1	—	—
10	BAAAABBBAAAAAAAA	—	—	1	—	—	2	—	1	—	—	—
11	BABAAAAAAAAAAABA	1	1	2	—	—	1	1	1	—	1	1
12	AAAAAAAAABACAAAA	—	—	—	—	—	—	—	1	—	2	—
13	AAAAAAAAABABAAAA	7	4	4	2	1	12	7	2	10	15	6
15	AAAAAAAAAAAABABA	—	—	1	—	—	1	—	1	—	—	—
17	AAAAABAABAAAAAAC	—	—	—	—	1	—	—	—	—	—	—
19	AAAAABACAAAAAAAA	—	—	—	—	1	—	—	—	—	—	—
20	AAAAAAAAAAAAAABB	—	—	—	—	1	—	—	—	—	—	—
21	AAAAABABAAAAAAAA	—	1	—	—	1	1	—	—	—	—	—
22	ABAACAAACBAAAABA	—	—	—	—	1	—	—	—	—	—	—
23	AABAABAAAAAAAAAA	3	1	2	—	1	1	—	—	—	—	—
24	BAAAAAAAAAAABABA	—	1	—	—	—	—	—	—	—	—	—
25	AAAAAAAAACABCAAA	—	—	—	—	—	—	—	—	2	—	—
26	BAAABAAAAAAAAAAA	—	1	—	—	—	—	—	—	—	—	—
27	ABAACAAACAAAACBA	1	—	—	—	—	—	—	—	—	—	—
28	AAAAAAAAABAAAAAA	—	2	—	—	—	—	1	—	—	—	—
29	AAAACAAAAAAAAABA	—	1	—	—	—	—	—	—	—	—	—
30	AAAABBAAAAAAACAA	—	—	1	—	—	—	—	—	—	—	—
31	AAAAABBAAAAAAAAA	—	1	—	—	—	—	—	—	—	—	—
32	AAAACAAAADDDAABD	—	1	1	—	—	—	—	—	—	—	—
33	AAABAAAAAAAAAAAA	1	—	—	—	—	1	—	—	—	—	—
34	AACAAAAAABABAAAA	2	—	—	—	—	—	—	—	—	—	—
35	AAAAABAAAAAAAACA	1	—	—	—	—	—	1	1	1	—	—
36	BABAABAAAAAAAABA	1	1	—	—	—	—	—	—	—	—	—
37	ABAACAAACAAAAABA	1	—	2	—	—	—	2	—	4	1	—
38	AAAAAAAAACABAAAA	1	—	—	—	—	1	—	—	—	1	—
39	AABACCAAADDDAABD	—	—	—	—	—	—	—	—	—	1	—
40	AAAAABAAAAAACAAA	—	—	—	—	—	—	1	—	—	1	—
41	ABAAAAAAABABAAAA	—	—	—	—	—	—	—	—	—	1	—
42	AAAAABAAAAAABAAA	—	—	—	—	—	—	—	—	1	—	—
43	AAAAABAAAAAAAAAE	—	—	—	—	—	1	—	—	—	—	—
44	AAAAABCAAAAAAABA	—	—	1	—	—	1	—	—	—	—	—
45	AAAAABCAAAAAAAAA	—	—	1	—	—	1	—	—	—	—	—
46	AAAACCAAADDDCABD	1	—	—	—	—	1	—	—	—	—	—
47	AAAAABAAAABAABAA	—	—	—	—	—	—	—	—	1	—	—
48	AAAAABAAAAAAACAA	1	2	—	—	—	—	2	—	2	1	—
49	AAAAABAAABABAAAA	—	—	1	—	—	—	—	—	—	—	—
50	AAAAABAAABAAAAAA	—	—	—	—	—	—	1	—	—	—	—
51	AAAACAAACAAAAABA	—	—	—	—	—	—	1	—	—	—	1
52	AABAABAAAABAABAA	—	—	—	—	—	—	—	—	—	—	1
53	AAAACAAAAAADAAAD	—	—	—	—	—	—	1	—	—	—	—

the percentage nucleotide-sequence divergence among the 49 haplotypes ranged from 0.156 to 2.623 (mean ±SE=0.980 ±0.015). MtDNA nucleon diversity was 0.905, and intrapopulational nucleotide sequence diversity was 0.548 ±0.412 (mean ±SD). The latter estimates were based on all 444 individuals surveyed. Nucleon diversity within samples (Table 3) ranged from 0.845 in the sample from Sarasota, Florida, to

Table 3

Mitochondrial (mt)DNA nucleon and intrapopulational nucleotide sequence diversities among samples of greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and Atlantic Ocean

Locality	Number of individuals	Number of haplotypes	Nucleon diversity	Nucleotide sequence diversity (±SD)[1]
Port Aransas, TX	58	17	0.886	0.561 ± 0.443
Freeport, TX	44	18	0.899	0.515 ± 0.387
Port Fourchon, LA	43	16	0.901	0.579 ± 0.435
Gulfport, MS	7	5	0.905	0.587 ± 0.415
Pensacola, FL	24	12	0.906	0.601 ± 0.449
Panama City, FL	50	17	0.872	0.525 ± 0.415
St. Petersburg, FL	42	15	0.879	0.519 ± 0.395
Sarasota, FL	32	12	0.845	0.483 ± 0.370
Florida Keys	55	14	0.893	0.619 ± 0.419
New Smyrna, FL	53	14	0.861	0.537 ± 0.427
South Carolina	36	10	0.846	0.502 ± 0.342
Total	444	49	0.905	0.548 ± 0.412

[1] In per cent.

0.906 in the sample from Pensacola, Florida. Intrapopulational nucleotide sequence diversity within samples (Table 3) ranged (mean ±SD) from 0.483 ±0.370 in the sample from Sarasota, Florida, to 0.619 ±0.419 in the sample from the Florida Keys. The latter values are all within one standard error (estimated from bootstrap analysis) of one another, indicating that levels of mtDNA variation are essentially identical throughout the geographic area surveyed. Levels of mtDNA variability, as measured by nucleon diversity and intrapopulational nucleotide sequence diversity, are commensurate with those estimated for several other marine fish of commercial or recreational value (Gold et al., 1993).

Results of bootstrap analyses and log-likelihood tests of spatial homogeneity in mtDNA haplotype frequencies are shown in Table 4. Tests were carried out 1) among all sample localities, 2) among samples from the Gulf, and 3) between defined groups where samples were pooled. In the last category, defined groups were based on region, i.e. Gulf and Atlantic. Gulf samples included the following: Port Aransas, TX; Freeport, TX; Port Fourchon, LA; Gulfport, MS; Pensacola, FL; Panama City, FL; and Sarasota, FL. Atlantic samples included New Smyrna, FL, and South Carolina. Two sets of pooled comparisons were carried out: in one, the sample from the Florida Keys was included with Gulf samples; in the other, the sample from the Florida Keys was included with Atlantic samples. We used this approach because the sample from the Florida Keys is located on the geographic boundary between the two putative stocks of greater amberjack (Cummings and McClellan[3]), and we could not place *a priori* the sample from the Florida Keys into either stock before testing the two-stock hypothesis. Significant heterogeneity was found only in the pooled comparison of samples from the Atlantic and Florida Keys versus samples from the Gulf; tests of homogeneity among all samples, among samples from the Gulf, and between pooled samples from the Atlantic versus those from the Gulf plus the Florida Keys were nonsignificant (Table 4). Estimates of F_{ST} revealed the same pattern; the F_{ST}

Table 4

Tests for spatial homogeneity in mtDNA haplotype frequencies among greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and U.S. southeastern Atlantic. F_{ST} is a measure of variance in haplotype frequencies. Number of samples is in parentheses.

Test group	Number of haplotypes	Homogeneity tests[1] P_{RB}	P_G	F_{ST}
All samples (11)	49	0.158	>0.050	0.005
Gulf samples (8)	45	0.250	>0.050	0.003
Pooled comparisons				
Gulf + Florida Keys vs. Atlantic (2)	49	0.627	>0.050	0.004
Atlantic + Florida Keys vs. Gulf (2)	49	0.042	~0.002	0.009[2]

[1] P_{RB} is probability from randomization (bootstrap) approach of Roff and Bentzen (1989); P_G is probability from log-likelihood (G) test (Sokal and Rohlf, 1969).
[2] Value differs significantly (P=0.007) from 0.00; all other F_{ST} values are nonsignificant.

Table 5

Hierarchical analysis of molecular variation (AMOVA) among mtDNA haplotypes of greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and U.S. southeastern Atlantic.

Variance component	Observed partition		P^1	Φ values
	Variance	% total		
Gulf + Florida Keys vs. Atlantic				
Between regions	0.00127	0.29	0.259	$\Phi_{CT} = 0.003$
Among samples within regions	0.00160	0.36	0.190	$\Phi_{SC} = 0.004$
Within samples	0.43974	99.35	0.121	$\Phi_{ST} = 0.006$
Atlantic + Florida Keys vs. Gulf				
Between regions	0.00398	0.90	0.001	$\Phi_{CT} = 0.009$
Among samples within regions	0.00011	0.03	0.444	$\Phi_{SC} = 0.000$
Within samples	0.43974	99.07	0.113	$\Phi_{ST} = 0.009$

[1] Probability of finding a more extreme variance component by chance alone (5000 permutations).

value of 0.009 in the pooled comparison of samples from the Atlantic and Florida Keys versus samples from the Gulf differed significantly from zero, whereas F_{ST} values in all other comparisons were nonsignificant (Table 4).

For AMOVA, where the variance in mtDNA haplotypes was partitioned by region, we also performed two separate analyses: one where the sample from the Florida Keys was included with Gulf samples, and one where the sample from the Florida Keys was included with Atlantic samples. In both analyses, the majority of the variance (>99%) was distributed within samples, and in both, the among-samples-within-groups component (Φ_{SC}) was nonsignificant (Table 5). The between-region component (Φ_{CT}) in the comparison of Atlantic and Florida Keys versus the Gulf was highly significant ($P<0.001$); whereas Φ_{CT} in the comparison of Atlantic versus the Gulf plus Florida Keys was not (Table 5). These results paralleled results of the homogeneity tests and the F_{ST} values. Finally, we employed pairwise Φ_{ST} values (i.e. between pairs of samples) to generate average "distance" values between samples from the Gulf (eight total), between samples from the Atlantic plus the sample from the Florida Keys (three total), and between samples from the Gulf versus samples from the Atlantic (and the Florida Keys). The average (±SE) Φ_{ST} values for these comparisons were 0.004 ±0.001 (samples from the Gulf), 0.002 ±0.002 (samples from the Atlantic and the Florida Keys), and 0.012 ±0.002 (samples from the Gulf versus samples from the Atlantic and the Florida Keys).

Homogeneity (V) tests were carried out on the ten haplotypes (1–4, 6, 11, 13, 23, 37, and 48) that were found in at least six individuals. Over all 11 samples, only haplotype 6 differed significantly (P~0.048) in frequency. This result is not significant when corrections for multiple testing (Rice, 1989) are applied. We then pooled samples into two groups (one that included the eight samples from the Gulf, and one that included the two samples from the Atlantic and the sample from the Florida Keys). Significant V tests (again, prior to corrections for multiple testing) were found for haplotype 6 (P~0.011) and haplotype 13 (P~0.024). Frequency plots by sample of these two haplotypes (Fig. 2) revealed a clinal pattern to variation in haplotype 6, with frequencies generally increasing eastwardly in the Gulf and into the Atlantic; no pattern was apparent for variation in haplotype 13.

Maximum-parsimony (MP) analysis of individual mtDNA haplotypes and neighbor-joining (NJ) analysis of the matrix of interpopulational (genetic) distances revealed no evidence of phylogeographic structure. MP analysis generated an unresolved multichotomous topology where a few clades of individual mtDNA haplotypes were supported at 90% or greater in bootstrap analysis (100 replicates). In each case, haplotypes within such clades were not geographically cohesive. Examples included a clade of haplotypes 5, 52, and 47, which were found, respectively, in waters off Texas and west Florida (5), South Carolina (52), and the Florida Keys (47) (Table 2); a clade of haplotypes 32, 39, and 46, which were found, respectively, in waters off Texas and Louisiana (42), east Florida (39), and Texas and west Florida (46) (Table 2); and a clade of haplotypes 22, 27, 37, and 51, which were found, respectively, in waters off west Florida (22), Texas (27), several localities (37), and west Florida and South Carolina (51) (Table 2). No "deep" subdivisions, in the sense of multiple restriction sites identifying a clade of haplotypes, were evident. The NJ topology (Fig. 3) indicated relatively

long terminal branches to individual samples, but short internodal branches linking samples. Samples did not necessarily cluster geographically, and in no case did geographically proximate samples form a cluster.

Spatial autocorrelation analyses generated 40 Moran's I values in each run (10 haplotypes × four distance classes). Four significant ($P<0.05$) values were obtained when equal numbers of pairwise comparisons were used: two were positive and occurred in the first and third distance classes, and two were negative and occurred in the third and fourth distance classes. Two significant values ($P<0.05$) were obtained when equal geographic distances were used: one in the first distance class was positive, and one in the third distance class was negative. Mean I values were negative in all four distance classes in both runs and did not differ significantly from expected values of I in the absence of autocorrelation (Fig. 4).

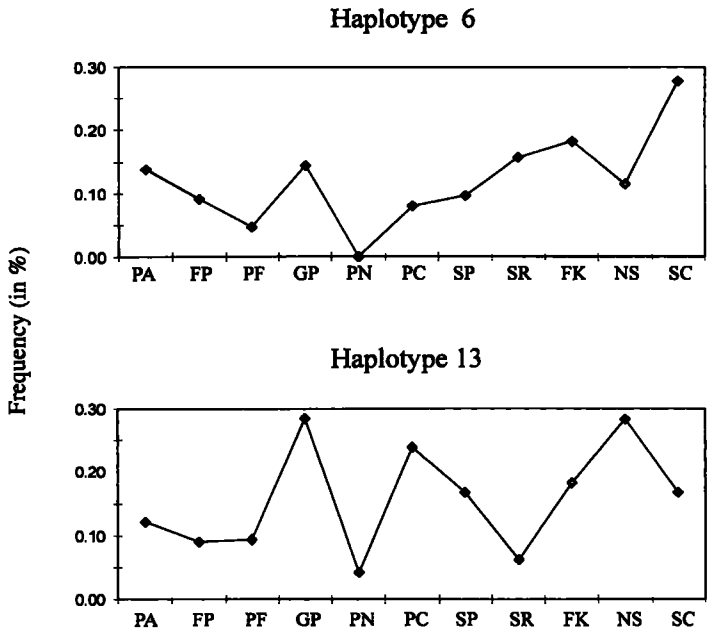

Figure 2

Frequency of mtDNA haplotypes 6 and 13 among greater amberjack (*Seriola dumerili*) sampled from 11 localities in the northern Gulf of Mexico and southeastern (U.S.) Atlantic. Acronyms are defined in Table 1.

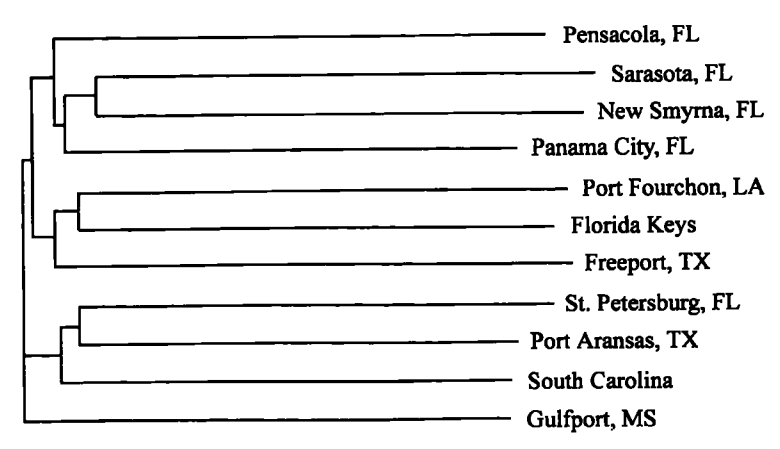

Figure 3

Neighbor-joining topology generated from matrix of pairwise, interpopulational (mtDNA) nucleotide sequence divergence among samples of greater amberjack, *Seriola dumerili*.

Discussion

Variation of mtDNA in greater amberjack is consistent with the hypothesis that one subpopulation (stock) exists in the northern Gulf of Mexico (Gulf), whereas a second subpopulation (stock) exists along the U.S. southeast Atlantic coast (Atlantic). The latter (Atlantic) subpopulation includes the sample from the Florida Keys. Evidence supporting this hypothesis includes 1) significant heterogeneity in the distribution of pooled haplotypes (and of haplotype 6) from the Gulf versus those from the Atlantic (and the Florida Keys); 2) a significant (nonzero) F_{ST} value in the same comparison; 3) a highly significant ($P<0.001$) between-region component (Φ_{CT} value) in AMOVA, when regions were defined as the Gulf versus the Atlantic (and the Florida Keys); and 4) comparison of pairwise Φ_{ST} "distances" where samples from the Gulf or from the Atlantic were at least three times more similar to one another than were samples from the Gulf versus those from the Atlantic (and the Florida Keys).

Neither maximum-parsimony (MP) analysis of individual mtDNA haplotypes nor neighbor-joining analysis of a matrix of interpopulational (intersample) nucleotide-sequence distances revealed phylogeographic patterns indicative of population structuring. A few, well-supported clades of individual haplotypes were detected in MP analysis, but in no case were haplotypes within individual clades from the same or geographically proximate

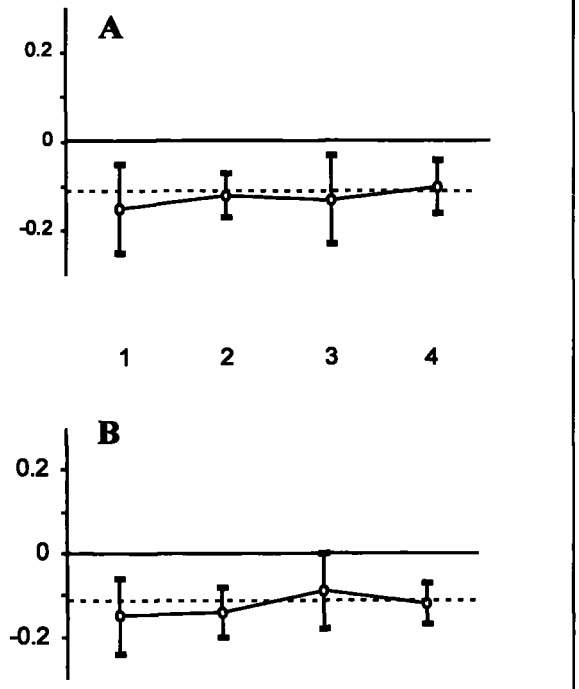

Figure 4

Correlograms based on frequencies of mtDNA haplotypes found in eight or more individuals among samples of greater amberjack, *Seriola dumerili*, from the northern Gulf of Mexico. Abscissas: distance classes 1–4 (left to right); ordinates: mean autocorrelation coefficients (Moran's *I* values) for each distance class (±SE). (**A**) Equal frequencies/distance class; (**B**) equal distances between distance classes.

sample localities. Absence of structure in MP analysis is not inconsistent with results of homogeneity testing, F_{ST} values, and AMOVA analysis, in that 1) presumed restrictions in gene flow between subpopulations could be relatively recent, i.e. there has been insufficient time for haplotypes (e.g. haplotype 6) that differ in frequency between subpopulations to become reciprocally monophyletic, or 2) there may be limited gene flow between subpopulations. Finally, there was no indication of spatial autocorrelation (positive or negative) in common haplotypes among samples from the Gulf. This finding indicates the absence of an isolation-by-distance effect among greater amberjack in the Gulf and is consistent with the hypothesis of continuous gene flow across the northern Gulf.

Divergence in mtDNA between Gulf and Atlantic subpopulations has been documented for a variety of marine species. In some (e.g. American oysters, toadfish, black sea bass, and to a lesser extent, horseshoe crabs), major phylogeographic discontinuities between Gulf and Atlantic subpopulations were found, leading to the hypothesis that the similar vicariant patterns may have stemmed from episodic changes in environmental conditions during Pleistocene glaciation (Avise, 1992). In addition, the presence of phylogeographic structure was taken to indicate that current-day gene flow between subpopulations is very restricted, if it occurs at all. In species such as red drum (Gold et al., 1993), king mackerel (Gold et al., 1997), and greater amberjack (our study), mtDNA differences between Gulf and Atlantic subpopulations are documented but are limited to either a frequency difference in a single mtDNA haplotype, a small (but significant) difference in mtDNA haplotype distribution, or both. The relatively small genetic differences observed between subpopulations in these species, along with the absence of phylogeographic structure, both between regions and among haplotypes, suggest either that limited gene flow occurs between subpopulations or that separation between subpopulations is fairly recent. In the case of greater amberjack, the former is consistent with mark-and-recapture data that suggest exchange rates of 1.5% between Gulf and Atlantic stocks (Cummings and McClellan[3]).

For greater amberjack, the boundary between Gulf and Atlantic subpopulations appears to be between the Florida Keys (included in the Atlantic subpopulation) and somewhere off the central-western Florida coast, possibly the Florida Middle Ground, a series of north-south reef structures located about 150 km south of the north Florida coast and about 160 km northwest of Tampa Bay (Hopkins et al.[6]), and the primary source for amberjack fishermen located in Sarasota and St. Petersburg. Separation between Gulf and Atlantic subpopulations (stocks) of greater amberjack could stem from a number of causes that involve historical or recent interactions between dispersal capability and impediments to gene flow, or both. Among present-day alternatives are 1) offshore currents that are not conducive to unrestricted movement between regions; and 2) absence of suitable habitat or difference in ecological (biogeographic) provinces between regions. A third (historical) possibility might be that subpopulations were separated (e.g. during Pleistocene glaciations) and have only recently (in geological time) begun exchanging genes. Rates of approach to genetic homogeneity under this last hypothesis are, in part, time-dependent, and one could speculate that there has been insufficient time for accumulated genetic differences to disappear.

[6] Hopkins, T. 1981. Florida Middle Ground. *In* R. Rezak and T. J. Bright (eds.), Final report: northern Gulf of Mexico topographic features study, p. 1–5. Tech. Rep. No. 81-2-T, Dep. Oceanography, Texas A&M University, College Station, TX.

There is at least suggestive evidence for each of the above three possibilities in greater amberjack. First, measurements of offshore current velocities are consistent with increased passive movement of individuals from the Florida Keys into the Atlantic rather than northward along the west Florida coast. Mean current velocities (145 m below the surface) eastward from the Keys through the Florida Straits are approximately 75 cm/sec, and along the southeast Florida coast, these currents can be as fast as 95 cm/sec (SAIC[7]). Because current velocities are expected to be greater nearer the surface, and because greater amberjack have been observed spawning in the Florida Keys (Cummings and McClellan[3]), buoyant eggs and larvae would likely be impacted and have little opportunity to move northward along the west coast of Florida. Second, in terms of habitat, the southern half of the Florida peninsula represents a transition zone between temperate and tropical forms, where the southern ranges of many temperate species terminate in tropical southern Florida (Briggs, 1974). There also is relatively little reef habitat or sharp topography between the Florida Middle Ground and the Keys; most of the area has a relatively smooth bottom comprising shell, sand, and quartz (Rezak et al., 1985; Rezak and Bright[8]). Because greater amberjack exhibit a marked preference for reefs, rock outcrops, and wrecks (Shipp, 1986; Manooch, 1988), it is possible that the paucity of significant reef or other major structure on the Outer Continental Shelf off southwestern Florida may inhibit movement of greater amberjack between the Florida Middle Ground and the Keys. Finally, although relatively little is known about the early life history of greater amberjack, spawning is thought to occur from mid-spring to early summer both along the southeastern coast of Florida (including the Florida Keys) and in the northern Gulf off Louisiana (Cummings and McClellan[3]; Thompson[9]). This spawning time suggests that warmer water temperatures may trigger onset of reproductive activity. One might then hypothesize that during the late Pleistocene Epoch, when waters of the northern Gulf were much cooler (Rezak et al., 1985), subpopulations of greater amberjack were isolated in warm-water refugia, perhaps off the Florida Keys (or in the Caribbean) and off the Yucatan Peninsula (Campeche Banks) where considerable reef habitat exists (Rezak et al., 1985). Following glacial retreat, the (putatively) isolated subpopulations could then have returned to the northern Gulf. Because the rate of approach to genetic homogeneity would be partly a function of time and gene flow, genetic differences between present-day subpopulations of greater amberjack could simply be historical artifacts, reflecting insufficient time (or restricted gene flow) relative to genetic homogenization.

Our finding that the sample of greater amberjack from the Florida Keys was included in a grouping (subpopulation) with samples from the Atlantic differs from a recent study in king mackerel (Gold et al., 1997), where a sample from the Florida Keys was placed in a grouping with samples from the Gulf. King mackerel are highly migratory, and the sample of king mackerel from the Florida Keys was taken during late winter when the majority of king mackerel in the Keys are thought to be from the Gulf Migratory Unit or stock (Williams and Godcharles[10]). The sample of greater amberjack from the Keys, however, was obtained during late March–early April, a time when the majority of king mackerel in the Keys are considered to be from the Atlantic Migratory Unit or stock (Williams and Godcharles[10]). Although greater amberjack are not migratory in the same way as king mackerel, it would be of more than passing interest to examine winter samples of greater amberjack from the Florida Keys and ask whether the Florida Keys constitute a mixing zone in greater amberjack as in king mackerel. Along these lines, it also would be important to examine both summer and winter samples of greater amberjack in the area between the Florida Middle Ground and the Florida Keys. A better definition of the geographic limits of the two subpopulations is critical for assessment and allocation of the greater amberjack resources along the west coast of Florida.

Avise et al. (1988) presented models that allow estimation of evolutionary (long-term) effective female-population size (N_{fe} values) based on mtDNA intrapopulational nucleotide sequence diversities. Assuming that the generation time in greater amberjack is three years (Wilson[11]), we estimated N_{fe} for the two subpopulations (stocks) of greater amberjack to be 90,000 (Gulf of Mexico) and 93,500 (U.S. southeastern Atlantic, including the Florida Keys).

[7] SAIC (Science Applications International Corporation). 1992. Straits of Florida physical oceanographic field study, final interpretative report, volume II: technical report. OCS Report/MMS 92-0024. U. S. Dep. Interior, Minerals Mgmt. Serv., Gulf of Mexico OCS Regional Office, New Orleans, LA, 179 p.

[8] Rezak, R., and T. J. Bright (eds.). 1981. Final report: northern Gulf of Mexico topographic features study. Tech. Rep. No. 81-2-T, Dept. Oceanography, Texas A&M University, College Station, TX, 150 p.

[9] Thompson, B. A. 1997. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803. Personal commun.

[10] Williams, R. O., and M. F. Godcharles. 1984. Completion report, king mackerel tagging and stock assessment. Project 2-341-R. Florida Dep. Natural Resources., St. Petersburg, FL.

[11] Wilson, C. A. 1997. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803. Personal commun.

The two estimates do not differ significantly from one another and are larger than those reported in a more limited (in terms of sample size and geographic coverage) study of greater amberjack (Richardson and Gold, 1993). They also are commensurate with $N_{f(e)}$ estimates for several other marine fish of commercial or recreational value (Gold et al., 1993). Although a positive correlation exists between effective population sizes and census sizes, the latter are generally an order of magnitude or two larger than the former (Avise et al., 1988). One reason for this difference, at least in estimates of $N_{f(e)}$ values for poikilothermic vertebrates, is that the models of Avise et al. (1988) employ (estimated) mtDNA evolutionary rates for homeothermic vertebrates. Estimated mtDNA evolutionary rates for poikilothermic vertebrates may be 5–10 times less than those for homeothermic vertebrates (Martin and Palumbi, 1993), suggesting that long-term effective population sizes of each subpopulation of greater amberjack could be on the order of 500,000 to 1,000,000 females.

According to results of this project, current stock boundaries for assessment and allocation of greater amberjack resources in U.S. waters appear appropriate except possibly for the west coast of Florida, south of the Florida Middle Ground. Depending on present or future importance of the greater amberjack fishery in this area, it would be useful to know both the geographic limits of the two subpopulations and whether the Florida Keys constitute a mixing zone for greater amberjack as for species such as king mackerel.

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Literature cited

Avise, J. C.
1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

Avise, J. C., R. M. Ball, and J. Arnold.
1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. 5:331–344.

Berry, F. H., and R. K. Burch.
1978. Aspects of the amberjack fisheries. Proc. Gulf Caribb. Fish. Inst. 31:179–194.

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

Briggs, J. C.
1974. Marine zoogeography. McGraw Hill, New York, NY, 475 p.

Camper, J. D., R. C. Barber, L. R. Richardson, and J. R. Gold.
1993. Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol. Mar. Biol. Biotechnol. 3:154–161.

DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston.
1987. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. Genetics 116:215–223.

Excoffier, L., P. E. Smouse, and J. M. Quattro.
1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Felsenstein, J.
1989. PHYLIP (Phylogeny Inference Package), version 3.4. Cladistics 5:164–166.

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

Gold, J. R., Á. Ý. Kristmundsdóttir, and L. R. Richardson.
1997. Mitochondrial DNA variation in king mackerel (*Scomberomorus cavalla*) from the western Atlantic Ocean and Gulf of Mexico. Mar. Biol. 129:221–232.

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

GMFMC (Gulf of Mexico Fishery Management Council).
1989. Amendment number 1 to the reef fish fishery management plan. Gulf of Mexico Fishery Management Council, Tampa, FL, 356 p.

Hilborn, R.
1985. Apparent stock-recruitment relationships in mixed stock fisheries. Can. J. Fish. Aquat. Sci. 42:718–723.

Manooch, C. S., III.
1988. Fisherman's guide: fishes of the southeastern United States. NC State Mus. Nat. Hist., Raleigh, NC, 362 p.

Martin, A. P., and S. R. Palumbi.
1993. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. (USA) 90:4087–4091.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield.
1992. REAP-The restriction enzyme analysis package. J. Hered. 83:157–158.

Nei, M., and J. C. Miller.
1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction site data. Genetics 125:873–879.

Nei, M., and F. Tajima.
1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

Rezak, R., T. J. Bright, and D. W. McGrail.
1985. Reefs and banks of the northwestern Gulf of Mexico. John Wiley & Sons, New York, NY, 259 p.

Rice, W. R.
1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Richardson, L. R., and J. R. Gold.
1993. Mitochondrial DNA variation in red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*) from the Gulf of Mexico. ICES J. Mar. Sci. 50:53–62.

Roff, D. A., and P. Bentzen.
1989. The statistical analysis of mitochondrial polymorphisms: chi-square and the problem of small samples. Mol. Biol. Evol. 6:539–545.

Saitou, N., and M. Nei.
1987. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Shipp, R. L.
1986. Dr. Bob Shipp's guide to the fishes of the Gulf of Mexico. 20th Century Printing Co., Mobile, AL, 256 p.

Sinclair, M., V. C. Anthony, T. D. Iles, and R. N. O'Boyle.
1985. Stock assessment problems in Atlantic herring (*Clupea harengus*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 42:888–898.

Smith, P. J., and Y. Fujio.
1980. Genetic variation in marine teleosts: high variability in habitat specialists and low variability in habitat generalists. Mar. Biol. 69:7–20.

Sokal, R. R., and F. J. Rohlf.
1969. Biometry: the principles and practice of statistics in biological research. Freeman and Sons, San Francisco, CA, 776 p.

Southern, E. M.
1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

Swofford, D. L.
1991. PAUP: Phylogenetic analysis using parsimony. Users manual. Illinois Nat. Hist. Surv., Champaign, IL, 179 p.

Waples, R. S.
1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400.

Wartenberg, D.
1989. SAAP: a spatial autocorrelation analysis program. Dep. Environ. Community Med., Robert Wood Johnson Medical School, Univ. of Medicine and Dentistry of New Jersey, Piscataway, NJ, 28 p.

Weir, B. S.
1990. Genetic data analysis. Sinauer Assoc., Inc., Sunderland, MA, 377 p.

Weir, B. S., and C. C. Cockerham.
1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.