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Mitochondrial DNA Diversity and Population Structure of Spotted Seatrout (*Cynoscion nebulosus*) in Coastal Waters of the Southeastern United States

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Restriction-site variation in mitochondrial (mt)DNA was examined among 470 spotted seatrout (*Cynoscion nebulosus*) sampled from eight localities in the northern Gulf of Mexico (Gulf) and two localities along the southeastern (Atlantic) coast of the United States. mtDNA fragment patterns generated 81 distinct mtDNA haplotypes (genotypes), three of which were found in 64% of the individuals assayed. Nucleotide sequence divergence among mtDNA haplotypes ranged from 0.148 to 1.808% and averaged (±SD) 0.676 ± 0.296%. Significant heterogeneity in mtDNA haplotype frequencies was detected between regions (i.e., Gulf vs Atlantic) and among sample localities from the northern Gulf. The latter appears to stem primarily (but not exclusively) from heterogeneity among samples from the western Gulf. Frequency plots of two of the common mtDNA haplotypes revealed strong east–west clines across the northern Gulf, with distinctive frequency discontinuities ("steps") between Gulf and Atlantic samples and between the westernmost sample in the Gulf (the lower Laguna Madre in Texas) and the next-most geographically proximate sample to the east. Spatial autocorrelation analysis of mtDNA haplotype frequencies among samples from the northern Gulf revealed a strong isolation-by-distance effect. These results support the hypothesis that spotted seatrout are spatially subdivided into discrete subpopulations or stocks. Divergence between Gulf and Atlantic subpopulations is likely related to historical vicariance, stemming from climatic changes occurring during glacial times. Other factors likely reduce present-day gene flow between subpopulations in the two regions. Divergence among subpopulations in the northern Gulf is more likely due to behavioral factors that limit (female) dispersal from a natal bay or estuary. The latter is consistent with studies of life history and returns from mark-and-recapture experiments. mtDNA diversity, an index of evolutionary effective (female) population size, also differed significantly among samples, with spotted seatrout from the lower Laguna Madre possessing appreciably reduced mtDNA diversity. This indicates a reduction in the effective number of females in the lower Laguna Madre and suggests that careful monitoring of spotted seatrout in the estuary may be warranted.

Spotted seatrout (*Cynoscion nebulosus*) are a vital fisheries resource in estuaries and bays of the northern Gulf of Mexico (Gulf) and along the southeastern (Atlantic) coast of the United States. Historically, the species supported both commercial and recreational fisheries, although recreational harvests, at least in the recent past, have been considerably larger than commercial harvests (Van Voorhees et al., 1992; National Marine Fisheries Service, 1993). Perceived declines in spotted seatrout in the Gulf have been attributed, in part, to loss of key habitat and overfishing (Shipp, 1986; Pattillo et al., 1997) and have prompted closure or restrictions of commercial harvests in most Gulf coast states. Regulations for recreational landings of spotted seatrout in Gulf waters are adjusted on a state-to-state basis and vary among states (Gulf States Marine Fishery Commission, 1993). Regulations within most Gulf and Atlantic coast states generally have been predicated on a single-stock model, where allocations are the same across bays and estuaries within a state. Management of spotted seatrout in Florida, however, is now regionally based within the state (Muller et al., 1997).

Previous studies relating to stock or population structure of spotted seatrout in the northern Gulf and Atlantic are equivocal. The general perception (Lorio and Perret, 1978; Helser et al., 1993) is that movement of spotted seatrout is restricted largely to natal estuaries and is based primarily on salinity changes or spawning activity. Tagging studies (Moffett, 1961; Overstreet, 1983; Baker and Matlock, 1993) have indicated very little to no movement of juveniles or adults, with the overwhelming majority of returns occurring within

25–30 miles (40–48 km) of the release site. In one study, however, a distance of over 300 miles (480 km) from the release site was recorded (Moffett, 1961). Iverson and Tabb (1962), alternatively, reported differences in growth rates among spotted seatrout sampled from several localities along the Gulf coast of Florida. They interpreted these findings as consistent with existence of discrete subpopulations. Murphy and Taylor (1994), however, also examined growth rates among spotted seatrout from Florida but hypothesized that observed differences among estuaries reflected environmental and fishing effects rather than division into discrete subpopulations (stocks).

Genetic studies on the issue of stock structure in spotted seatrout also are equivocal. Weinstein and Yerger (1976) surveyed spotted seatrout sampled from seven estuaries on the west (Gulf) coast of Florida and one from the east (Atlantic) coast for variation in general protein banding patterns. They reported discrete banding patterns in fish from each estuary, indicative of discrete subpopulations, and, moreover, that genetic similarity decreased with increased geographic distance between estuaries. Ramsey and Wakeman (1987) surveyed allelic variation at 40 presumptive (allozyme) loci among spotted seatrout from 13 localities in the northern Gulf and two along the eastern coast of Florida. Although spatial heterogeneity among localities was observed at three loci, overall subpopulation divergence was low (F_{ST} = 0.032) and strong regional or locality divergence was not indicated. They did, however, find spatial clumping of rare alleles and an isolation-by-distance effect (the latter suggested by observed patterns of spatial autocorrelation of alleles). Similar results, i.e., significant spatial heterogeneity at an allozyme locus (aspartate aminotransferase), low allele diversification (overall F_{ST} = 0.012), and an isolation-by-distance effect based on spatial autocorrelation analysis, were found by King and Pate (1992) in their study of 44 presumptive (allozyme) loci among spotted seatrout from 12 localities along the Gulf coast of Texas and northern Mexico. King and Pate (1992) hypothesized that a westerly directed, nearshore transport mechanism (primarily of eggs and larvae) facilitated exchange of spotted seatrout genes among Texas estuaries. This hypothesis was consistent with known life history in that spotted seatrout eggs and larvae are largely pelagic, whereas juveniles and adults are largely demersal (Pattillo et al., 1997).

In this study, we addressed the issue of stock structure among spotted seatrout in the northern Gulf and Atlantic through analysis of restriction site variation in mitochondrial (mt)DNA. Our objective was to test the null hypothesis that spotted seatrout in the southeastern United States comprise a single stock or subpopulation. We sampled extensively within Texas to ask whether genetic (mtDNA) variation was partitioned spatially within a smaller geographic region. The issue of stock structure (or geographic definition) is of importance when conducting stock assessments (Hilborn, 1985; Sinclair et al., 1985), and use of mtDNA to identify subdivision within marine species is documented (Avise, 1987; Ovenden, 1990; Gold and Richardson, 1998a). An overview of much of the data in this paper was published previously (Gold and Richardson, 1998a) as part of an international symposium on the conservation and genetics of marine organisms.

Methods

A total of 470 spotted seatrout was sampled by angling between 1989 and 1991 from eight localities in the northern Gulf and two localities along the southeastern (U.S.) Atlantic coast; sample sizes per locality ranged from 25 in Aransas Bay, TX, to 83 in Matagorda Bay, TX (Table 1; Fig. 1). Tissues (primarily heart, spleen, and white muscle) were removed from individual specimens, placed in cryopreservation tubes, quick-frozen in liquid nitrogen, and transported to College Station where they were stored at −80 C in an ultracold freezer.

Methods used to assay variation in restriction enzyme sites in mtDNA followed those outlined in Gold and Richardson (1991). Twelve restriction enzymes (*Apa*I, *Bgl*II, *Eco*RV, *Hin*dIII, *Nhe*I, *Nsi*I, *Pst*I, *Sca*I, *Spe*I, *Ssp*I, *Stu*I, and *Xmn*I) were used to digest 1.0–1.5 μg of DNA in 40-μl reactions according to manufacturer's specifications. Southern transfer, hybridization to an mtDNA probe, and autoradiography were used to identify fragments. The probe (pSOmt4) used was a 9.0–9.2-kilobase (kb) fragment of mtDNA from a related sciaenid, the red drum (*Sciaenops ocellatus*). Lambda DNA digested with *Hin*dIII was used as a molecular weight marker on each gel, and mtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII migration distances. All restriction sites detected in spotted seatrout mtDNA were mapped with single and double digestions or inferred from fragment patterns. A total of 83 mtDNA restriction sites was surveyed. Single-digestion mtDNA fragment pat-

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TABLE 1. Sample localities, number of individuals assayed, and intrapopulational mtDNA diversity of spotted seatrout from the northern Gulf of Mexico and the southeastern U.S. Atlantic coast.

Locality	Number of individuals	Number of haplotypes	Intrapopulational mtDNA diversity (mean ± SD)
Northern Gulf of Mexico			
Lower Laguna Madre, TX	55	16	0.236 ± 0.229
Aransas Bay, TX	25	6	0.357 ± 0.317
Matagorda Bay, TX	83	31	0.433 ± 0.315
Galveston Bay, TX	34	16	0.506 ± 0.326
Sabine Pass, TX	31	13	0.430 ± 0.312
Grand Isle, LA	47	14	0.447 ± 0.325
Apalachicola Bay, FL	54	18	0.454 ± 0.327
Tampa Bay, FL	50	15	0.414 ± 0.292
Southeast U.S. Atlantic coast			
Mosquito Lagoon, FL	41	10	0.444 ± 0.369
Bulls Bay, SC	50	12	0.414 ± 0.351

terns used to identify mtDNA haplotypes, the restriction site presence/absence matrix used to generate estimates of nucleotide sequence divergence, and the spatial distribution of the 81 mtDNA haplotypes revealed by single-digestion patterns are available from the second author.

Analysis of restriction site data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Nucleotide sequence divergence among mtDNA haplotypes was estimated after Nei and Li (1979), and intrapopulational nucleotide sequence diversity, the average nucleotide sequence divergence (difference) between any two individuals drawn at random from a given sample, was estimated after Nei and Tajima (1981). We refer to the latter as mtDNA diversity. Homogeneity of mtDNA diversity estimates among sample localities was tested by a Monte Carlo randomization procedure as described in Gold et al. (1999). Briefly, 100 randomized data sets were constructed by first pooling all mtDNA haplotypes and then allocating n haplotypes at random (with replacement) to 10 groups (the number of sample localities), where n was equal to observed sample sizes in each group (sample locality). Single classification (Sokal and Rohlf, 1966) and Kruskal–Wallis (Siegel, 1956) analyses of variance (ANOVAs) were carried out on the observed data and on each randomized replicate. We then compared F and chi-square statistics generated from the ANOVAs (based on observed data) to the distribution of F and chi-square values from randomized data sets. Significant heterogeneity at $\alpha = 0.05$ was indicated if observed values exceeded 95% of randomized values.

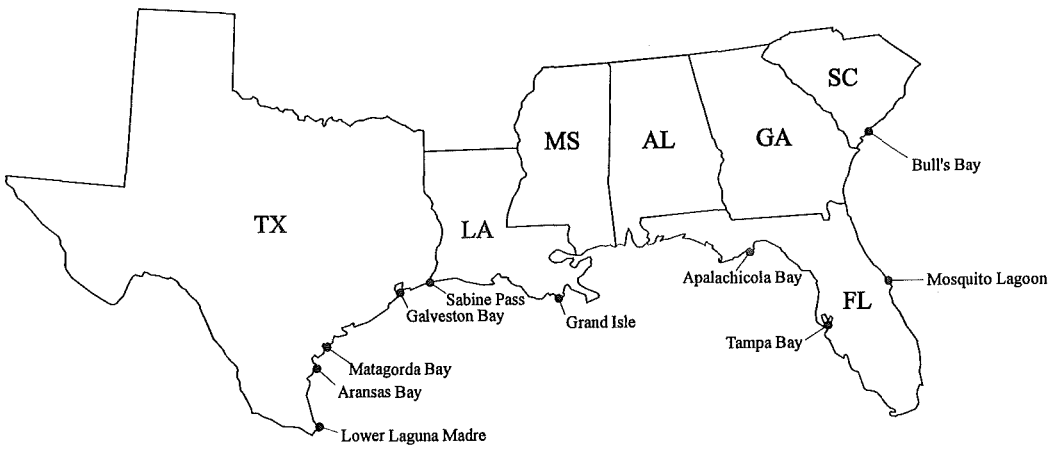

Fig. 1. Sampling localities for spotted seatrout, *Cynoscion nebulosus*, examined in the present study.

Significance testing of mtDNA haplotype frequencies among localities was carried out with the randomization (Monte Carlo) procedure of Roff and Bentzen (1989) and log-likelihood (G) tests (Sokal and Rohlf, 1966). Tests were organized (i) among all localities (10 total), (ii) among localities in the Gulf (eight), (iii) between localities in the Atlantic (two), (iv) between localities in the eastern Gulf (two), and (v) among localities in the western Gulf (six). We also tested homogeneity of mtDNA haplotype frequencies between pooled localities in the Gulf vs pooled localities in the Atlantic and between pooled localities in the eastern Gulf vs pooled localities in the western Gulf. Homogeneity of frequencies of single haplotypes (found in seven or more individuals) was examined via the V-test, employing arcsine, square root-transformed haplotype frequencies (DeSalle et al., 1987). Significance levels for multiple tests carried out simultaneously were adjusted by the sequential Bonferroni approach (Rice, 1989). We also used analysis of molecular variance (AMOVA) (Excoffier et al., 1992) to test homogeneity of mtDNA haplotype distributions across localities. This approach generates estimates of genetic variance components and Φ-statistics, a set of hierarchical F-statistic analogs. Significance of Φ-statistics was tested by random permutation (1,000 replicates). We organized sample localities for input into AMOVA in the same way as used for significance testing of mtDNA haplotype frequencies. In comparisons between regions, i.e., Gulf vs Atlantic and eastern Gulf vs western Gulf, hierarchical analysis permitted further testing of homogeneity among localities within regions. Finally, nucleotide sequence divergence among samples (interpopulational divergence) was estimated after Nei and Tajima (1981). Similarity analysis of the matrix of interpopulational divergence values employed the neighbor-joining algorithm of Saitou and Nei (1987) in version 3.4 of the Phylogeny Inference Package (PHYLIP) of Felsenstein (1992).

Spatial autocorrelation analysis of frequencies of common mtDNA haplotypes was used to examine whether haplotype frequencies at a sample locality were independent of haplotype frequencies at adjacent localities. The analysis was limited to the eight sample localities in the Gulf. We used the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989) and computed autocorrelation coefficients (Moran's I values) as a function of geographic distance between localities. "Noise" generated by low-frequency mtDNA haplotypes was eliminated by generating autocorrelation coefficients only for haplotypes found in ten or more individuals (five haplotypes total). The first of two SAAP runs employed equal geographic distances between each of four distance classes; the second employed equal numbers of pairwise comparisons in each distance class. The numbers of pairwise comparisons in the former were 9, 7, 4, and 8; the number of pairwise comparisons in the latter was 7. Distance classes in both runs were generated by SAAP from input longitude and latitude of each locality.

Results

mtDNA fragment patterns produced by single digestions with the 12 restriction enzymes generated 81 mtDNA haplotypes among 470 spotted seatrout assayed. Of 81 mtDNA haplotypes, three were found in 64% of the individuals surveyed: haplotype 1 (80 individuals), haplotype 2 (116 individuals), and haplotype 3 (106 individuals). Of the remaining haplotypes, two were found in 11–20 individuals, five were found in 5–8 individuals, and 20 were found in 2–4 individuals. Fifty-one haplotypes were unique to single individuals. The percentage of nucleotide sequence divergence among the 81 haplotypes ranged from 0.148 to 1.808 and averaged (± SD) 0.676 ± 0.296. The overall nucleon diversity (probability that any two individuals drawn at random will differ in mtDNA haplotype) was 0.857, and the overall intrapopulational mtDNA diversity (the average nucleotide sequence difference, in percent, between any two individuals drawn at random) was 0.452 ± 0.325 (SD).

Intrapopulational mtDNA diversity among samples ranged from 0.236 ± 0.229 (SD) in the sample from the lower Laguna Madre to 0.506 ± 0.326 in the sample from Galveston Bay. Significant heterogeneity ($P < 0.001$) of mtDNA diversity values among samples was indicated by both single classification and Kruskal–Wallis analysis of variance. On the basis of the distribution of mtDNA diversities among samples (Table 1), the heterogeneity in mtDNA diversity appears to stem in large part from the low mtDNA diversity in the sample from the lower Laguna Madre.

Both permutation (bootstrap) and log-likelihood (G) tests revealed significant heterogeneity over all localities, in pooled comparisons of localities in the Gulf vs those from the Atlantic, and in pooled comparisons of localities in the eastern Gulf vs those in the western Gulf (Table 2). Tests of spatial homogeneity among eight Gulf localities and among six localities in

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TABLE 2. Tests of spatial homogeneity in mtDNA haplotype frequencies between and among samples of spotted seatrout from the northern Gulf of Mexico and the southeastern U.S. Atlantic coast.

Test group	Number of samples	Probability[a] P_{RB}	Probability[a] P_G
All localities	10	0.000	<0.001
Gulf localities	8	0.210	≈0.018
Atlantic localities	2	0.149	>0.050
East Gulf localities[b]	2	0.419	>0.050
West Gulf localities[c]	6	0.378	≈0.017
Pooled comparisons			
Gulf vs Atlantic	2	0.000	<0.001
East vs west Gulf	2	0.017	≈0.001

[a] P_{RB} = probability based on bootstrap analysis, 1,000 replicates (after Roff and Bentzen, 1989); P_G = probability based on log-likelihood (G) test (after Sokal and Rohlf, 1966).
[b] East Gulf localities: Tampa Bay, FL; Apalachicola Bay, FL.
[c] West Gulf localities: Lower Laguna Madre, TX; Aransas Bay, TX; Matagorda Bay, TX; Galveston Bay, TX; Sabine Pass, TX; Grand Isle, LA.

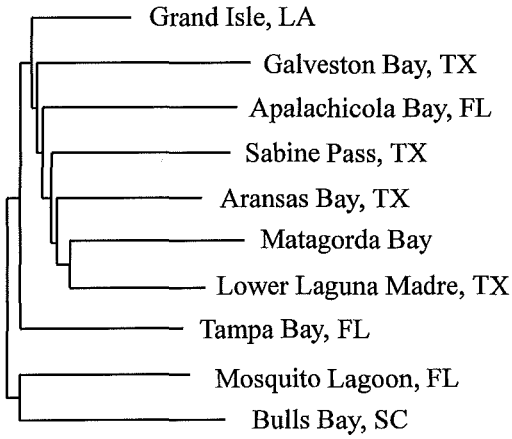

Fig. 3. Neighbor-joining topology generated from matrix of interpopulational (mtDNA) nucleotide sequence divergence values among samples of spotted seatrout.

the western Gulf were nonsignificant (permutation approach) or marginally so (log-likelihood approach, with adjusted α of 0.01 after Bonferroni correction). In general, pooled comparisons are expected to be more robust because of the increased sizes per sample cell. V-tests (DeSalle et al., 1987) of individual haplotypes found in more than 10 individuals (five haplotypes total) revealed significant spatial heterogeneity ($P < 0.05$) in frequencies of haplotypes 1 ($P < 0.001$, adjusted α of 0.01) and 3 ($P \approx 0.006$, adjusted α of 0.013). Frequency plots of haplotypes 1 and 3 (Fig. 2) revealed a strong east–west cline across the northern Gulf, along with a frequency discontinuity between Gulf and Atlantic localities and between the westernmost sample in the Gulf (the lower Laguna Madre) and the next-most geographically proximate sample from Aransas Bay. The distinction between localities in the Gulf vs those in the Atlantic also is evident in the neighbor-joining topology (Fig. 3) derived from the matrix of interpopulational nucleotide sequence divergence.

AMOVA revealed a pattern similar to that indicated by homogeneity tests (Table 3). Significant Φ values ($P < 0.001$ in each case) were found in comparisons among all 10 localities, among eight localities in the Gulf, and among six localities in the western Gulf. In hierarchical tests, the between-region Φ value for the Gulf vs Atlantic comparison was significant ($P = 0.019$), whereas that for the east vs west Gulf comparison was not ($P = 0.178$). However, in both hierarchical tests, Φ values for the proportion of the variance attributable to among localities within regions were significant ($P < 0.001$ in the Gulf vs Atlantic comparison, and $P = 0.002$ in the east vs west Gulf comparison). Collectively, results of the spatial homogeneity tests, V-tests, and AMOVA indicate significant

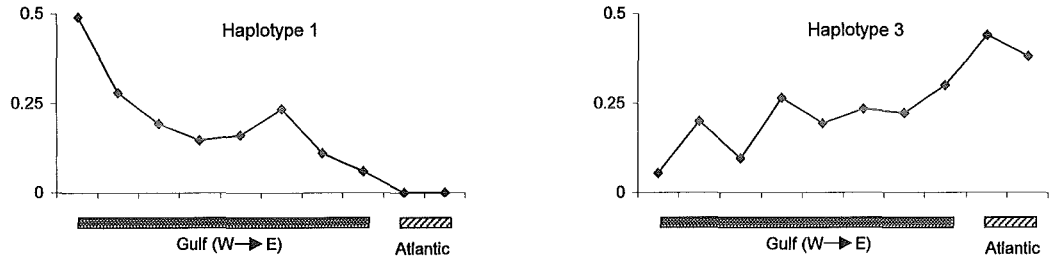

Fig. 2. Frequencies of haplotypes 1 and 3 in spotted seatrout, *Cynoscion nebulosus*, in the northern Gulf of Mexico and along the southeastern Atlantic coast of the United States. Abscissa: geographic localities range (left to right) from the lower Laguna Madre (TX) to Tampa Bay (FL) in the Gulf of Mexico, and from Mosquito Lagoon (FL) to Bulls Bay (SC) in the Atlantic.

TABLE 3. Analysis of molecular variation (AMOVA) among mtDNA haplotypes of spotted seatrout from the northern Gulf of Mexico and the southeastern U.S. Atlantic coast.

Variance component	Observed partition		ϕ value	P^a
	Variance	% Total		
All localities				
Among localities	0.01827	4.26	0.043	<0.001
Within localities	0.41039	95.74	—	—
Gulf localities				
Among localities	0.01172	2.74	0.027	<0.001
Within localities	0.41611	97.26	—	—
Atlantic localities				
Between localities	0.00028	0.07	0.001	0.387
Within localities	0.38655	99.93	—	—
East Gulf localities[b]				
Between localities	0.00028	0.07	0.001	0.301
Within localities	0.38655	99.93	—	—
West Gulf localities[b]				
Among localities	0.01044	2.47	0.025	<0.001
Within localities	0.41309	97.53	—	—
Gulf vs Atlantic				
Between regions	0.02542	5.70	0.057	0.019
Among localities within regions	0.01037	2.32	0.025	<0.001
Within localities	0.41339	91.98	—	—
East Gulf vs west Gulf[b]				
Between regions	0.01041	2.40	0.024	0.178
Among localities within regions	0.00820	1.89	0.019	0.002
Within localities	0.41611	95.72	—	—

[a] Probability of finding a more extreme variance component by chance alone (1,000 permutations).
[b] East and west Gulf localities as in Table 2.

differences in mtDNA haplotype frequencies between spotted seatrout in the Gulf vs those in the Atlantic and among samples from the Gulf. The latter appears to stem primarily (but not exclusively) from heterogeneity among samples in the western Gulf, with noticeable differences occurring in spotted seatrout sampled from the lower Laguna Madre. Except for the comparison between the Gulf and Atlantic, where 5.7% and 2.3% of the variance were attributable to between regions and among localities within regions, respectively, more than 95% of the variance in mtDNA haplotype frequencies was distributed within sampling localities (Table 3).

Spatial autocorrelation analysis of mtDNA haplotype frequencies among sample localities in the northern Gulf revealed an isolation-by-distance effect where haplotype frequencies were positively correlated in proximal sampling localities and negatively correlated in distal sampling localities. Of 20 Moran's I values obtained in SAAP runs when equal distances between distance classes were used, two significant values (both positive) were found in the first two distance classes, and four significant values (all negative) were found in the last two distance classes. Nearly identical results were obtained in SAAP runs with equal number of pairwise comparisons in each distance class (three of four significant values in the first two distance classes were positive, whereas all three significant values in the last two distance classes were negative). Correlograms (Gulf samples only) of mean ($\pm$ SE) Moran's I values in both SAAP runs (Fig. 4) indicate a steady decline from positive autocorrelation at 400–500 km between sample localities to significant negative autocorrelation at >1,000 km.

DISCUSSION

The pattern of genetic divergence among spotted seatrout in the southeastern United States parallels that found in several marine fish species: regionally distinct subpopulations

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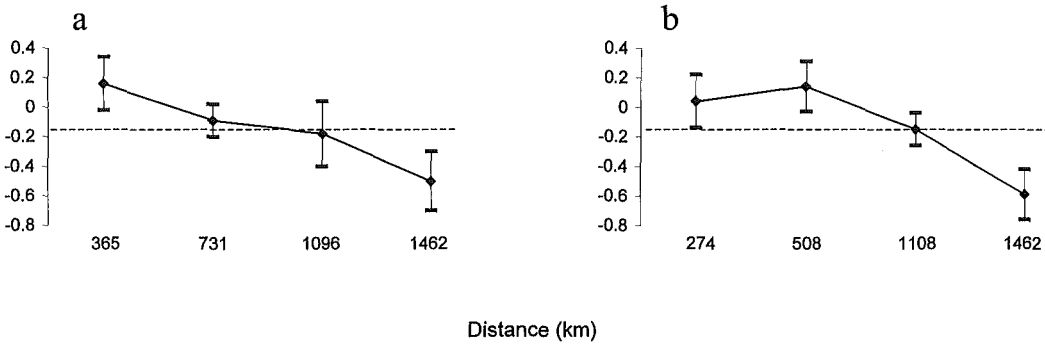

Fig. 4. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in spotted seatrout from the Gulf of Mexico: (a) equal distance between distance classes, (b) equal number of comparisons per distance class. Abscissa: distance classes (four total). Ordinate: mean autocorrelation coefficients (Moran's I values) for each distance class. Bars about each mean represent 1 SE on either side of a mean. Dashed line is expected Moran's I value when no correlation exists.

occur in the northern Gulf of Mexico (Gulf) and along the southeastern (U.S.) Atlantic coast (Atlantic). Virtually all between-region comparisons of mtDNA haplotype frequencies in spotted seatrout data were significant, and more than 5% of the total variance in mtDNA haplotypes was distributed between the two regions. Other marine finfish where distinct subpopulations occur in the Gulf and Atlantic include toadfish, black sea bass, greater amberjack, red drum, and black drum (Avise, 1992; Gold and Richardson 1998a, 1998b). Avise (1992) hypothesized that the shared patterns of regional divergence among the different species reflected similar vicariant histories that likely were related to climatic changes occurring during glacial times. Other factors affecting present-day populations likely include (i) absence of suitable habitat at the spatial junction between subpopulations, and (ii) oceanographic currents that minimize movement from the Atlantic into the northern Gulf relative to movement in the reverse direction (Gold and Richardson, 1998a, 1998b). Whether the latter affected or contributed to initial subdivision is problematic; however, both would reduce gene flow between present-day subpopulations in each region. Occurrence of an isolation-by-distance effect among samples of spotted seatrout (see below) also indicates that female behavior (natal philopatry or limited dispersal from a natal bay or estuary) may play a role in minimizing present-day gene exchange between spotted seatrout in the two regions. Our findings differ from those of Ramsey and Wakeman (1987), who were unable to detect a strong between-region component in spotted seatrout in their analysis of genetic variation at 40 presumptive allozyme loci. They did, however, find spatial clumping of low frequency or rare alleles and significant heterogeneity at three loci.

We also detected spatial heterogeneity in mtDNA haplotype frequencies among samples of spotted seatrout from the northern Gulf. Significant differences were detected in pooled comparisons between samples from the western Gulf and samples from the eastern Gulf, in the among-localities component of the AMOVA of Gulf localities, and in the among-localities-within-regions component of the hierarchical AMOVA in the western vs eastern Gulf comparison. Much of the heterogeneity in mtDNA haplotypes in the northern Gulf appears to be attributable to differences among localities in the western Gulf, as evidenced by significant heterogeneity detected in AMOVA of western but not eastern Gulf localities. Finally, two of three common haplotypes in spotted seatrout exhibited strong east–west clines in frequency across the northern Gulf, with notable "steps" occurring between the sample from the lower Laguna Madre (TX) and its nearest neighbor (Aransas Bay, TX). Significant genetic heterogeneity within the Gulf also was observed by Weinstein and Yerger (1976) in their analysis of general proteins among spotted seatrout from different estuaries on the west (Gulf) coast of Florida and by King and Pate (1992) among samples from the Gulf coast of Texas and northern Mexico. Weinstein and Yerger (1976) interpreted their data to indicate the existence of discrete subpopulations. King and Pate (1992), alternatively, interpreted the low overall F_{ST} value of 0.012 and the relatively high (>>>1) estimate of the genetic effective number of migrants (N_em) to indicate high gene flow throughout the study

area. Ramsey and Wakeman (1987) also detected significant allele frequency heterogeneity among their samples of spotted seatrout but, similar to King and Pate (1992), felt that the overall F_{ST} value (0.032) did not indicate strong subpopulational divergence. All three prior genetic studies of spotted seatrout, however, did detect an isolation-by-distance effect, and King and Pate (1992) reported a strong east–west cline in allele frequency at a locus for the enzyme aspartate aminotransferase.

In our view, genetic data accumulated to date are consistent with the hypothesis that spotted seatrout in the northern Gulf are spatially subdivided into discrete subpopulations or stocks. First, results of our study indicate significant differences in mtDNA haplotype frequencies both across the northern Gulf and among localities sampled in the western Gulf. In general, the expectation is that mtDNA is more sensitive than allozymes as a means to detect population subdivision. This is due in part to the haploid, uniclonal (maternal) inheritance of mtDNA and in part to a more rapid rate of DNA sequence evolution relative to analogous (i.e., protein coding) sequences in the nucleus (Brown, 1983; Wilson et al., 1985). Because of the former, effective population sizes needed to detect population subdivision and gene flow are at least four times smaller for mtDNA than for nuclear genes (Birky et al., 1983; Templeton, 1987), and because of the latter, mtDNA would be more sensitive to events occurring more recently in historical time. Second, the allozyme data of Ramsey and Wakemen (1987) and King and Pate (1992) did reveal heterogeneity in allelic frequencies among samples of spotted seatrout. However, in both studies, the authors placed more credence in measures of population subdivision (F_{ST}) and gene flow (N_em) than in tests of allele frequency homogeneity. In our study, Φ values (which are analogous to F_{ST}) derived from AMOVA were 0.027 Gulfwide and 0.025 in the western Gulf. These Φ values are nearly the same as the F_{ST} values reported in the allozyme studies, yet both Φ values were very highly significant ($P < 0.001$) in permutation tests. As pointed out by Wright (1969) and Allendorf and Phelps (1981), significant heterogeneity in allele frequencies and the existence of population structure are not necessarily inconsistent with gene flow; small values of F can be associated with genetic divergence, even when substantial gene exchange occurs. Finally, all four genetic studies of spotted seatrout have detected an isolation-by-distance effect where dispersal of genes is inversely related to geographic distance. In general, isolation-by-distance effects can stem either from natal site philopatry (i.e., homing) or from limited movement of spawning adults away from a natal bay or estuary. In spotted seatrout, adult life history and mark-and-recapture data (Moffett, 1961; Overstreet, 1983; Pattillo et al., 1997) are more consistent with the latter than with the former. In either case, the operative biological mechanism is behavioral, although physiological traits also may impact the actual distance migrated (Roff, 1991). An important point to note is that an isolation-by-distance effect in spotted seatrout has been found for both nuclear genes and mtDNA, indicating that the presumed behavioral patterns occur in both sexes. The implication of these considerations is that current management of spotted seatrout as a single stock within the borders of most Gulf coast states may not be warranted.

We also found a significant difference in intrapopulational mtDNA diversity among the samples of spotted seatrout. On the basis of observed estimates of mtDNA diversity, we inferred minimally that mtDNA diversity was reduced significantly in the sample from the lower Laguna Madre in Texas. Briefly, mtDNA diversity is the average genetic difference between any two individuals drawn at random from a sample and represents an index of evolutionary effective (female) population size or $N_{f(e)}$ (Avise et al., 1988; Ball et al., 1990) when mtDNA evolutionary rate and generation time are the same across samples. Given that our samples of spotted seatrout are conspecific, we assume the latter to be the case and that the heterogeneity in mtDNA diversity implies significant differences in $N_{f(e)}$ among the samples in our study.

In general, effective population size (N_e) provides an estimate of the degree to which genetic drift can change gene (allele) frequencies and the rate(s) of allelic fixation (i.e., loss of genetic diversity) due to genetic drift (Hartl and Clark, 1989). It also is an approximate estimate of the number of effective breeding individuals in a subpopulation (Waples, 1990). $N_{f(e)}$ thus is a measure of the potential change in mtDNA haplotype frequency and an approximate estimate of the number of spawning females. The latter is of interest to management of a fishery because even approximate estimates of the number of spawning females could relate to critical fishery measures such as spawning potential ratio. The implication from our data is that spotted seatrout in the lower Laguna Madre may be at risk, relative to spotted seatrout in nearby bays or estuaries, to a

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population decline because of reduced genetic diversity. This implication follows from suggestions that the magnitude of genetic variability may influence the probability of survival of a population over ecological and/or evolutionary time (Vrijenhoek et al., 1985; Quattro and Vrijenhoek, 1989).

A constraint to the above is that $N_{f(e)}$ can reflect "historical" demographics that may not necessarily represent present-day conditions. Studies comparing $N_{f(e)}$ estimates with estimates of present-day census size, for example, often reveal the former to be at least an order of magnitude smaller than the latter (Avise, 1992). This usually is attributed to (i) slower rates of mtDNA evolution than are typically employed when estimating $N_{f(e)}$, or (ii) decreases (bottlenecks) in the number of females through which mtDNA lineages are or have been transmitted (Avise, 1992). The reverse, i.e., where patterns of mtDNA diversity indicate large effective population sizes but present-day census does not, also has been reported (Lavery et al., 1996). In both cases, the central issue is whether the genetic signal represents a present-day or historical change in demography that affected effective population size. Unfortunately, we cannot address this issue directly regarding spotted seatrout in the lower Laguna Madre. We do not have reliable estimates of mtDNA evolutionary rate in spotted seatrout with which to estimate effective (female) population size that then could be compared with present-day census sizes. We also do not have historical samples that could be used to examine whether effective population sizes have increased or decreased over time. However, given our assumption that mtDNA evolutionary rate is the same across samples, the reduction in mtDNA diversity in spotted seatrout from the lower Laguna Madre strongly indicates a reduction in the effective number of females. If such a reduction has occurred in recent generations, more careful monitoring of the lower Laguna Madre spotted seatrout fishery would be warranted. We are currently beginning assays of microsatellite loci in spotted seatrout to ask whether differences occur in patterns of hypervariable nuclear genes as well.

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