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SEDAR74-RD57

March 2021

ELSEVIER

Fisheries Research 20 (1994) 137–150

FISHERIES RESEARCH

Mitochondrial DNA variation among 'red' fishes from the Gulf of Mexico[1]

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(Accepted 15 March 1994)

Abstract

Restriction-site variation in mitochondrial (mt)DNA was used to assess population (stock) structure in three species of 'red' fishes from the Gulf of Mexico (Gulf): red drum (*Sciaenops ocellatus*), red snapper (*Lutjanus campechanus*), and red grouper (*Epinephelus morio*). MtDNA haplotype frequencies among geographic samples of all three species were statistically homogeneous, and phylogenetic analysis and phenetic clustering of mtDNA haplotypes or of geographic samples in each species revealed no evidence of phylogeographic cohesion. These findings are consistent with the hypothesis that each species is composed of a single, panmictic population in the Gulf. Autocorrelations of mtDNA haplotype frequencies in red drum were positive in proximal distance classes and negative in distal distance classes, indicating that migration of red drum within the Gulf is inversely related to geographic distance from an estuary or bay of natal origin. Estimates of intrapopulational mtDNA nucleotide sequence diversity differed significantly among geographic samples in all three species. The differences were especially pronounced among samples of red snapper and red grouper. In red drum and red snapper, intrapopulational mtDNA diversities appear temporally stable. Spatial differences in intrapopulational mtDNA diversity imply geographic differences in effective female population size and may signal relevant units for management of fishery resources.

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[1]This paper was adapted from a presentation given at the International Workshop on Ecosystem Function of Marine Biodiversity in Estuaries, Lagoons and Near-shore Coastal Ecosystems, held in Gosier, Guadalupe, in March 1993. A shorter version of this paper will appear in the proceedings of the workshop, to be published in a special issue of *Biology International*.

0165-7836/94/$07.00
SSDI 0165-7836(94)00290-D

1. Introduction

Effective management of fishery resources requires critical information on the population or stock structure of the exploited species. This is especially true for marine fisheries, in part because physical impediments to gene flow (potentially leading to allopatric diversification) are often lacking, and in part because jurisdictional boundaries for resource allocation frequently overlap. Historically, information on stock structure of economically-important fishes was derived largely from morphological and/or life-history data (Ihssen et al., 1981). The use of genetic data to examine stock structure, however, has shown that many fish populations are substructured despite morphological or other, non-genetic evidence that indicated the occurrence of only a single stock (Berst and Simon, 1981; Allendorf et al., 1987). Conversely, there also are examples where multiple stocks were assumed on the basis of morphology, geography, spawning location, and/or behavior, but where genetic data have indicated the occurrence of only a single stock (Kornfield and Bogdanowicz, 1987; Avise et al., 1987; Martin et al., 1992).

In this paper, we present data on genetic variation among three species of 'red' fishes from the Gulf of Mexico (Gulf). The species are the red drum (*Sciaenops ocellatus*), the red snapper (*Lutjanus campechanus*), and the red grouper (*Epinephelus morio*). All three support important commercial and/or recreational fisheries in the Gulf and all three are regulated in response to overfishing and perceived population declines. Because recreational harvests of red drum occur primarily in estuaries and nearshore waters, regulations in the Gulf are set by individual states, i.e. red drum are managed as separate units. Red snapper and red grouper, alternatively, are harvested primarily in the Gulf of Mexico Exclusive Economic Zone and adjoining Territorial Sea, and are considered a unit stock for management purposes (Gulf of Mexico Fishery Management Council (GMFMC), 1989, 1991). The focus of our research has been to determine whether genetic variation is partitioned spatially in each species (i.e. whether genetically meaningful management units exist within the Gulf). An additional objective has been to determine whether levels of genetic variation differ either within or among each species. Much of our work is published (Richardson and Gold, 1993; Camper et al., 1993; Gold et al., 1993) and has been condensed for presentation here.

The three 'red' fish species differ in life-history parameters affecting the potential for gene flow and population structuring. Red drum (family Sciaenidae) are largely estuarine dependent as larvae and juveniles, whereas mature adults are found offshore in large schools that can migrate extensively (Matlock, 1987). Red snapper (family Lutjanidae) are sedentary and non-migratory as adults, typically preferring low- and high-relief hard bottoms (Bradley and Bryan, 1974; Fable, 1980). Juvenile red snapper also appear to be substrate specific, whereas red snapper eggs and larvae are pelagic (Bradley and Bryan, 1974). Red grouper (family Serranidae) are also pelagic as larvae, and unlike most groupers, are not restricted to the immediate vicinity of reef ledges (Bullock and Smith, 1991). Adult red grouper, however, are generally concentrated around reef or reef-like

structures, including ledges, crevices, and shipwrecks (Manooch, 1988). Previous genetic studies of red drum (Ramsey and Wakeman, 1987) and red snapper (Johnson, 1987) have not revealed significant population substructuring in either species within the Gulf.

The genetic assay used in our work is restriction-enzyme site variation in mitochondrial (mt)DNA. Briefly, mtDNA in vertebrates is a physically circular, easily identified molecule that is uniclonally inherited through the maternal parent (Wilson et al., 1985). Because mtDNA is genetically haploid and mtDNA variants do not segregate and recombine during sexual reproduction, the effective population sizes needed to evaluate population subdivision and gene flow are in theory four times less for mtDNA than for nuclear genes (Birky et al., 1983). MtDNA also appears to have a rapid rate of sequence evolution in vertebrates (Brown, 1983; Wilson et al., 1985), which means that mtDNA should be useful in identifying events of recent origin.

2. Materials and methods

Localities in the Gulf from which individuals have been sampled are shown in Fig. 1. The number of individuals sampled at each locality is given in the figure legend. Ages of individual red drum were determined from annuli on otoliths. Ages of red snapper and red grouper were not determined. Red snapper were

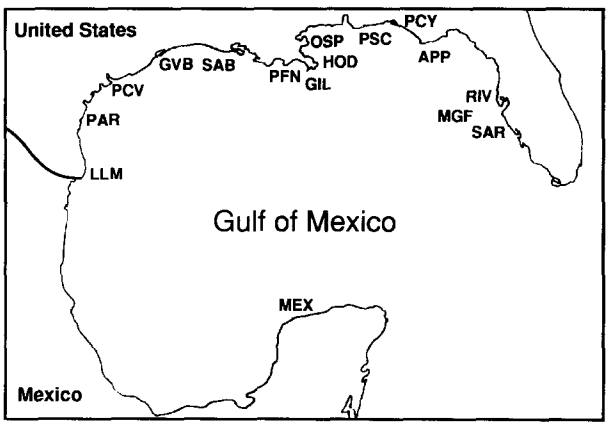

Fig. 1. Localities in the Gulf of Mexico from which samples of red drum, red snapper, and red grouper were procured. The number of individuals sampled at each locality is given in parentheses. Abbreviations refer to localities as follows: red drum (SAR—Sarasota Bay, Florida (111); RIV—Riviera Bay, Florida (69); APP—Apalachicola Bay, Florida (67); OSP—Biloxi Bay, Florida (117); HOD—Black Bay, Louisiana (20); GIL—Grand Isle, Louisiana (90); SAB—Sabine Pass, Texas (43); GVB—West Bay, Texas (68); PCV—Pass Cavallo, Texas (31); PAR—Redfish Bay, Texas (38); LLM—Lower Laguna Madre, Texas (39)); red snapper (PSC—Pensacola, Florida (25); PCY—Panama City, Florida (50); PFN—Port Fourchon, Louisiana (36); PAR—Port Aransas, Texas (25 in 1990 and 35 in 1991)); red grouper (MGF—Middle Grounds, Florida (46); MER—Merida, Mexico (48)).

sampled in 1990 and 1991 from the northwest coast of Florida (Pensacola and Panama City) and from Port Aransas, Texas, to carry out temporal comparisons of mtDNA haplotype frequencies and intrapopulational mtDNA diversities. Details of tissue removal and storage and of the assay of individual mtDNA restriction sites may be found in the primary papers.

Spatial partitioning of mtDNA variation in each species was assessed by heterogeneity testing of mtDNA haplotype frequencies among sample localities and by searches for phylogeographic cohesion of mtDNA haplotypes using phyletic and phenetic approaches. Heterogeneity tests have included (1) log-likelihood (G) tests, (2) a Monte Carlo randomization procedure developed by Roff and Bentzen (1989), and (3) V tests of common mtDNA haplotypes (DeSalle et al., 1987). The Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1991) was used for maximum-parsimony analysis of restriction site presence–absence matrices, and the unweighted pair-group method using arithmetic

Table 1
Synopsis of mtDNA data from three species

Parameter	Species		
	Red drum	Red snapper	Red grouper
Number of individuals assayed	693	171	94
Number of mtDNA restriction sites assayed	104	76	115
Number of mtDNA haplotypes	99	41	16
Nucleon diversity	0.95	0.78	0.41
Nucleotide sequence divergence among mtDNA haplotypes[a]	0.88	0.50	0.28

[a]In percent; estimated using equations of Nei and Li (1979).

Table 2
Results of tests for spatial partitioning of mtDNA variation among samples of three species from the Gulf of Mexico

Test group	Number of localities	Number of haplotypes tested	Number of significant V tests	P[a]	Results of G tests	F_{ST}	$N_e m_f$
Red drum (1986)[b]	11	24	1[c]	0.032	$P>0.05$	−0.002	>10
Red drum (1987)[b]	10	20	1[c]	0.408	$P<0.02$	0.008	>10
Red snapper	5	7	0	0.615	$P>0.05$	−0.001	>10
Red grouper	2	2	0	0.586	$P>0.05$	−0.009	>10

F_{ST} is a measure of the variance in mtDNA haplotype frequencies and was estimated using formulae of Weir and Cockerham (1984). $N_e m_f$ is the effective number of female migrants per generation and was estimated using Wright's (1943) island model modified for mtDNA (i.e. $F_{ST}=1/(2N_e m_f+1)$).
[a]Non-significant ($P>0.05$) when corrected for multiple tests.
[b]Red drum are from the 1986 and 1987 year classes.
[c]P, probability based on 1000 bootstrap replications (Roff and Bentzen, 1989).

averages (UPGMA) algorithm was used for phenetic clustering of nucleotide sequence divergence (distance) matrices. Minimum-length parsimony networks of mtDNA haplotypes were constructed by connecting composite mtDNA genotypes in increments of single gains or losses of restriction sites. For spatial autocorrelation analysis of frequencies of common mtDNA haplotypes, the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989) was employed.

MtDNA variation in each species was assessed by nucleon diversity (the probability that any two individuals drawn at random will differ in mtDNA haplotype) and by intrapopulational nucleotide sequence diversity (the average nucleotide difference between any two individuals drawn at random). Both estimates of mtDNA variation were generated using equations of Nei and Tajima (1981). Homogeneity of intrapopulational nucleotide sequence diversities among (or be-

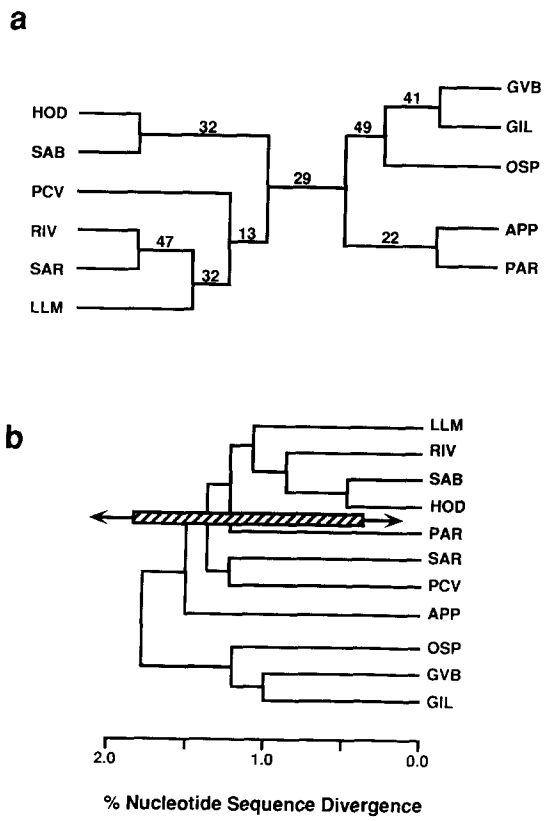

Fig. 2. (a) Strict (unrooted) consensus tree produced by maximum-parsimony analysis of a presence–absence restriction-site matrix of red drum samples. Numbers along branches indicate the proportion of times that a branch (clade) was distinguished in 500 boot-strap replicates. Branch lengths are not accurate representations of the number of character state changes. (b) UPGMA cluster analysis of percentage nucleotide sequence divergence among samples of red drum. Hatched bar is the standard error of the node it overlies. Abbreviations for samples are given in Fig. 1.

tween) samples in each species was tested using single classification analysis of variance (or Student's t test).

3. Results

A synopsis of the mtDNA survey in each species is given in Table 1. Red drum are by far the most variable in mtDNA, whereas red grouper are the least variable. On average, the probability that any two individuals drawn at random will differ in mtDNA haplotype is 95% (red drum), 78% (red snapper), and 41% (red grouper). In addition, individual mtDNA haplotypes found in red drum are more different from one another than are individual mtDNA haplotypes in red snapper and red grouper.

Results of tests for spatial heterogeneity in mtDNA haplotype frequencies are presented in Table 2. Data for red drum are shown by year class. Following cor-

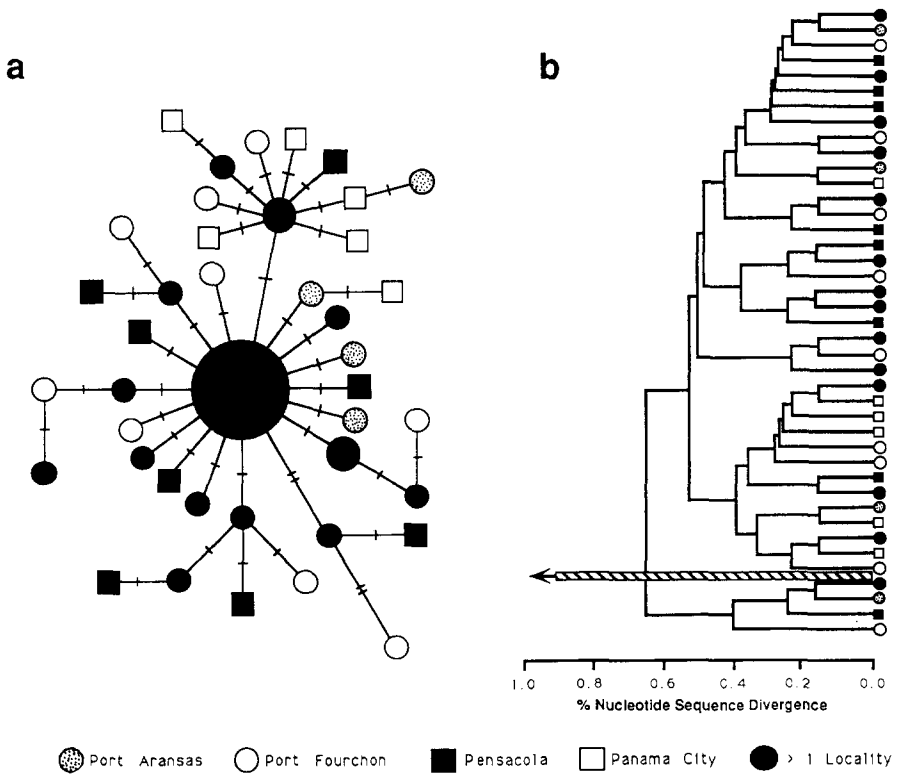

Fig. 3. (a) Parsimony network of 41 mtDNA haplotypes found in red snapper. Branches connecting haplotypes are drawn proportional to the number of restriction site changes (hatch marks) required to connect adjacent haplotypes. (b) UPGMA cluster analysis of percentage nucleotide sequence divergence values among 29 mtDNA haplotypes found in red snapper. Hatched bars are standard errors of the nodes they overlie.

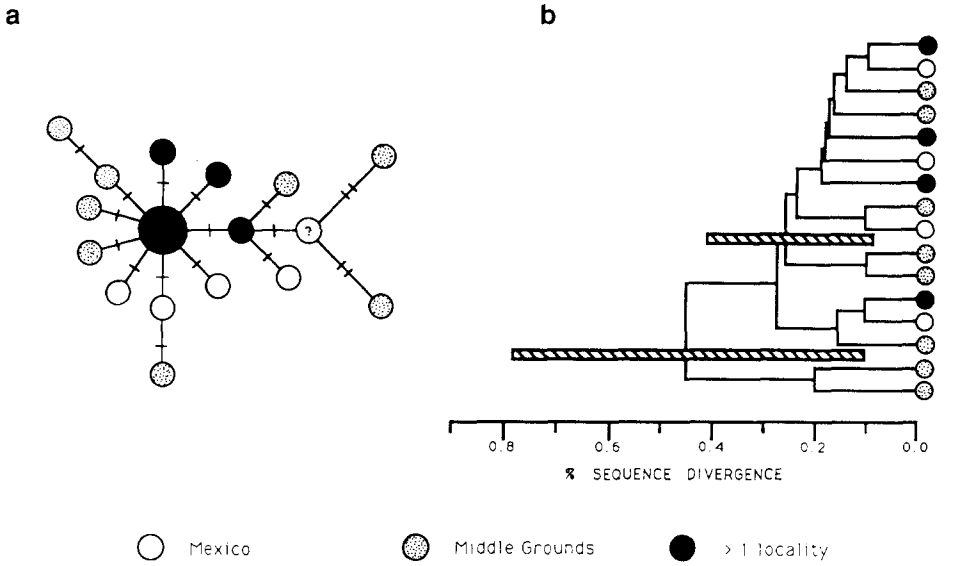

Fig. 4. (a) Parsimony network of 16 mtDNA haplotypes found in red grouper. Branches connecting haplotypes are drawn proportional to the number of restriction site changes (hatch marks) required to connect adjacent haplotypes. One haplotype (indicated by '?') was not found but was assumed to exist. (b) UPGMA cluster analysis of percentage nucleotide sequence divergence values among 16 mtDNA haplotypes found in red grouper. Hatched bars are standard errors of the nodes they overlie.

rections for multiple tests performed simultaneously (Cooper, 1968), no significant V tests were found among or between samples in any of the species. The Monte Carlo randomization procedure (after Roff and Bentzen, 1989) yielded a significant result ($P=0.032$) among samples from the 1986 year class of red drum, and a significant G value ($P<0.02$) among samples from the 1987 year class of red drum. No other significant test results were obtained. 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) indicate little genetic subdivision and the occurrence of significant gene flow among or between samples in each species (Table 2). Tests for temporal heterogeneity of mtDNA haplotype frequencies were carried out in red drum between samples from different year classes at ten of the localities and in red snapper between samples taken in 1990 and 1991 from Port Aransas, Texas, and from northwestern Florida (Pensacola and Panama City). No significant heterogeneity was detected in any of the temporal comparisons.

Maximum-parsimony analysis and phenetic clustering of the red drum mtDNA haplotype presence–absence and nucleotide sequence divergence matrices, respectively, revealed no evidence of geographic cohesion or phylogeographic structuring among mtDNA haplotypes. The strict consensus tree produced from the matrix of red drum samples (Fig. 2(a)) revealed no evidence of phyletic cohesion among geographically proximate samples, and in the UPGMA-derived phenogram of red drum samples (Fig. 2(b)), none of the branch lengths were

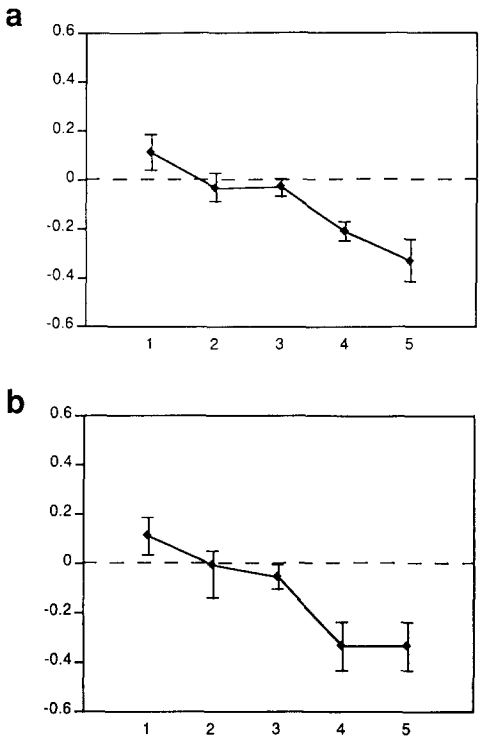

Fig. 5. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in red drum. Abscissae: distance classes 1–5 (left to right); ordinates: mean autocorrelation coefficients (Moran's *I* values) for each distance class. Bars about each mean value represent one standard error on either side of a mean. (a) Equal distances between distance classes; (b) equal frequencies/distance class.

significant as judged by standard errors of the most distant nodes. One of many possible minimum-length parsimony networks of the 41 mtDNA haplotypes found in red snapper (Fig. 3(a)) required a minimum of 42 steps. None of three approaches, i.e. the minimum-length network, a UPGMA-derived phenogram (Fig. 3(b)), or a strict consensus tree (not shown) of red snapper mtDNA haplotypes revealed any indication of phylogeographic cohesion of mtDNA haplotypes. Similar results (i.e. no evidence of phylogeographic structure) were obtained from analysis of red grouper mtDNA haplotypes (Fig. 4).

Spatial autocorrelation analysis of red drum mtDNA haplotype frequencies using both equal distances between distance classes (Fig. 5(a)) and equal frequencies of pairwise comparisons between localities (Fig. 5(b)) revealed positive autocorrelation in the first distance classes, no autocorrelation in the second and third distance classes, and negative autocorrelation in the last two distance classes. These results indicate that mtDNA haplotype frequencies in red drum are independent in all but geographically proximal localities.

Table 3
Per cent mtDNA intrapopulational nucleotide sequence diversities (±SD) among samples of three species from the Gulf of Mexico

Locality	Intrapopulational mtDNA nucleotide sequence diversity
Red drum	
RIV	0.480±0.280
OSP	0.536±0.297
GVB	0.542±0.295
SAB	0.573±0.301
SAR	0.581±0.284
APP	0.588±0.320
HOD	0.588±0.274
LLM	0.602±0.274
GIL	0.613±0.330
PAR	0.651±0.329
PCV	0.677±0.334
Red snapper	
PAR-90	0.140±0.135
PAR-91	0.179±0.162
PFN	0.282±0.210
PSC	0.311±0.214
PCY	0.277±0.199
Red grouper	
MGF	0.086±0.123
MEX	0.047±0.064

Intrapopulational mtDNA nucleotide sequence diversities for individual sample localities in each species are shown in Table 3. For red drum, individuals from the 1986 and 1987 year classes were pooled at each sample locality, as Student's t tests revealed no significant difference in intrapopulational mtDNA diversities between year classes at any locality. Significant heterogeneity ($P<0.05$) in intrapopulational mtDNA nucleotide sequence diversity was found among samples of all three species: red drum ($F_{(10,\ 26732)}=52.8$), red snapper ($F_{(4,\ 3045)}=63.6$), and red grouper ($t_{(2162)}=8.72$). Among samples of red drum, intrapopulational mtDNA diversities were continuously distributed, with a difference between high and low values of roughly 40%. In both red snapper and red grouper, the difference between high and low estimates was nearly twofold. In red snapper, intrapopulational mtDNA diversities from samples taken in different years from Port Aransas, Texas, and from northwestern Florida (Pensacola in 1990 and Panama City in 1991) did not differ significantly ($t_{(893)}=0.98$ and $t_{(1523)}=0.68$, respectively). Based on theoretical studies by Avise et al. (1988) and the assumption that mtDNA mutation rate is the same within each species, the differences in intrapopulational mtDNA diversities possibly reflect differences in the effective size of adult female populations that gave rise to each sample.

4. Discussion

The identification of spatially-distinct genetic units in strictly marine fish species has not proven a simple task. Previous genetic studies in a variety of species with significant pelagic stages in their life histories have typically revealed homogeneity in nuclear-gene and/or mtDNA allele frequencies over broad geographic areas. Examples include milkfish from the Pacific Ocean (Winans, 1980), skipjack, albacore, and yellowfin tuna from the Atlantic and Pacific Oceans (Graves et al., 1984; Graves and Dizon, 1989; Scoles and Graves, 1993), and Atlantic and Pacific herring (Grant, 1984; Grant and Utter, 1984; Kornfield and Bogdanowicz, 1987; Schweigert and Withler, 1990). Similar results (i.e. genetic homogeneity) also have been documented in numerous demersal species that have pelagic eggs and/or larvae. Examples include walleye pollock from the Bering Sea and the Gulf of Alaska (Grant and Utter, 1980; Mulligan et al., 1992), pink snapper from the Hawaiian Archipelago (Shaklee and Samollow, 1984), Atlantic cod (Mork et al., 1985; Smith et al., 1989), Pacific ocean perch (Seeb and Gunderson, 1988), and armorhead (Martin et al., 1992). In all of these studies, observed genetic homogeneity was interpreted as support for the hypothesis that each species was composed of a single, genetically panmictic unit and that gene flow (migration) was sufficient among localities to preclude significant genetic differentiation.

Recently, Avise and co-workers (Avise et al., 1987; Avise, 1992) have used a phylogenetic approach to search for population structure by asking whether intraspecific genetic diversity is non-randomly partitioned across geographic space. This approach employs intraspecific phylogenies of genetic characters to determine whether monophyletic assemblages occupy distinct regions within the range of a species, and has the advantage over homogeneity tests of allele frequencies that low-frequency alleles can contribute significantly to elucidation of geographic subdivision. In this way, Avise and co-workers have documented spatial subdivision in several vertebrate and invertebrate marine species (references in Avise, 1992).

In our studies of 'red' fishes from the Gulf of Mexico, both homogeneity tests of allele (mtDNA haplotype) frequencies and phylogenetic analysis of mtDNA lineages revealed no evidence of spatial–genetic subdivision in any of the three species within the Gulf. Based on contemporary approaches, these findings would be consistent with the hypothesis that each species was composed of a single, randomly mating population (stock) in the Gulf. For red drum, a single-stock hypothesis is compatible with life history in that adult red drum are primarily found in offshore waters and are known to migrate extensively within the Gulf (Matlock, 1987). In red snapper and red grouper, however, juveniles and adults are thought to be more sedentary and non-migratory (Bradley and Bryan, 1974; Manooch, 1988), with the pelagic phase of the life-cycle being restricted primarily to eggs and larvae.

Camper et al. (1993) pointed out four caveats to the single-stock hypothesis derived from genetic uniformity and/or absence of phylogeographic structure.

First, genetic homogeneity is simply consistent with a single-stock hypothesis, not unequivocal 'proof' that a single stock exists. The difficulty is that it is not easy to prove a null hypothesis. Second, observed genetic homogeneity may reflect historical rather than present-day events. Populations could be substructured, but have had enough genetic contact in the recent past to preclude significant genetic diversification. Third, populations could be substructured, but there could be low levels of current-day gene flow such that allele frequencies could not be distinguished via statistical analysis. Finally, in situations where one allele predominates across a population, substructuring would be difficult to detect if effective sizes of subpopulations were sufficiently small to continually promote high frequencies of a common allele through random drift effects.

In red drum, spatial autocorrelation analysis was used to examine independence of mtDNA haplotype frequencies as a function of increasing distance among sample localities. Autocorrelations of mtDNA haplotype frequencies were positive in proximal localities and negative in distal localities. This pattern of spatial autocorrelation indicates that mtDNA haplotype frequencies at distal localities are independent and is consistent with an 'isolation-by-distance' effect (Sokal and Oden, 1978) where gene flow is inversely related to geographic distance. The pattern also suggests that red drum in the Gulf are at least semi-isolated spatially, but that overall gene flow within the Gulf may be sufficient to neutralize genetic differentiation and phylogeographic structuring. A similar situation may occur among haddock from the western North Atlantic where reduced gene flow and discrete stocks were inferred from correlations between genetic dissimilarity and geographic distance (Zwanenburg et al., 1992). Both red drum and haddock could exemplify the third caveat listed above, where low levels of current-day gene flow preclude straightforward discrimination of genetic stocks. Spatial autocorrelation analysis of mtDNA haplotype frequencies in red snapper and red grouper may also prove informative once additional samples are procured.

Estimates of intrapopulational mtDNA diversity differed significantly among geographic samples in all three 'red' fish species. The differences were especially pronounced in red snapper and red grouper. Comparisons between different year classes of red drum sampled at the same locality and between samples of red snapper taken in different years in the same geographic area were non-significant. The spatial differences in intrapopulational mtDNA diversity imply significant differences in effective size of female populations that gave rise to individual samples. This implication is based on (1) theoretical studies by Avise et al. (1988), who showed that intrapopulational mtDNA diversities are directly proportional to effective sizes of female populations, and (2) the assumption that mtDNA sequence evolution and species generation time are constant among geographic samples within each species. In red drum, the observed temporal stability of intrapopulational mtDNA diversities at individual localities is consistent with the hypothesis that spatial differences in effective female population size occur across the Gulf. We are further testing this hypothesis by assaying mtDNA among red drum from the 1988 and 1989 year classes.

In red snapper and red grouper, the approximately twofold differences in intra-

populational mtDNA diversity between or among localities clearly imply the existence of spatial differences in effective female population size, and moreover, demonstrate that spatial units can be distinguished within a fishery, even if heterogeneity tests of allele frequencies and phylogeographic analysis of genetic characters fail to demonstrate the existence of distinct 'genetic' stocks. In this case, spatial units may reflect differences in local effective population size, a parameter of potential interest to management of the fishery. Whether the observed differences in intrapopulational mtDNA diversity signal different, isolated stocks of red snapper and red grouper is unknown. In both species, one mtDNA haplotype predominates at all sample localities, and the intrapopulational mtDNA diversities are among the lowest reported to date for marine fish species (Camper et al., 1993). This suggests that both species may be examples of the case where substructuring exists, but is difficult to detect because of small effective population size effects that result in high frequencies of a common allele and removal of low-frequency alleles.

Acknowledgments

The research has been supported by the Texas A&M University Sea Grant College Program (Awards NA85AA-D-SG128, NA89AA-D-SG139, and NA16RGO457-01), the MARFIN Program of the US Department of Commerce (Awards NA89-WC-H-MF025, NA90-AA-H-MF107, and NA90-AA-H-MF755), the Coastal Fisheries Branch of the Texas Parks and Wildlife Department, and the Texas Agricultural Experiment Station (Project H-6703). The views expressed in the paper are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or any of its subagencies. This paper represents number XII in the series 'Genetic Studies in Marine Fishes' and Contribution 30 of the Center for Biosystematics and Biodiversity at Texas A&M University.

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