

# Genetic Homogeneity among Geographic Samples of Snappers and Groupers: Evidence of Continuous Gene Flow?

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## ABSTRACT

We examined variation in mitochondrial (mt)DNA among samples of red snapper (*Lutjanus campechanus*) and red grouper (*Epinephelus morio*) from localities in the northern and western Gulf of Mexico. In both species, mtDNA haplotype frequencies were homogeneous between or among samples and there was no evidence of phylogeographic structure of haplotypes. In red snapper where multiple samples were examined, rare haplotypes were not clustered geographically and spatial autocorrelation of common haplotypes did not differ significantly from those expected when no correlation exists. These results are consistent with the hypothesis that gene flow between or among localities in both species is essentially continuous. The sedentary nature of juveniles and adults of both species suggests that gene flow may occur via hydrodynamic transport of pelagic eggs and larvae. Caveats to this hypothesis are considered. Levels of mtDNA variability, especially in red grouper, are among the lowest reported for marine fishes, and suggest minimally that genetic bottleneck events have occurred in the past or recent history of both species.

KEY WORDS: Genetics, groupers, snappers

## INTRODUCTION

Red snapper (*Lutjanus campechanus*) and red grouper (*Epinephelus morio*) are species of considerable economic importance in the Gulf of Mexico. Both species support commercial and recreational fisheries (Moe, 1969; Goodyear and Phares, 1990), and in recent years, perceived declines in abundance have led to regulation of commercial and recreational harvests of both species in U.S. waters. Red snapper and red grouper fisheries in Mexican waters remain essentially unregulated.

Studies in our laboratory over the past decade have focused on the issue of population (stock) structure in a variety of marine fishes in the Gulf of Mexico, including red snapper (Camper *et al.*, 1993; Gold *et al.*, 1997) and red grouper (Richardson and Gold, 1993, 1997). Knowledge of population structure in fisheries is critical for at least two reasons. The first is that different stocks, should they exist, may possess novel characteristics that contribute to long-term adaptability and survival at the metapopulation or species level (Stepien 1995).

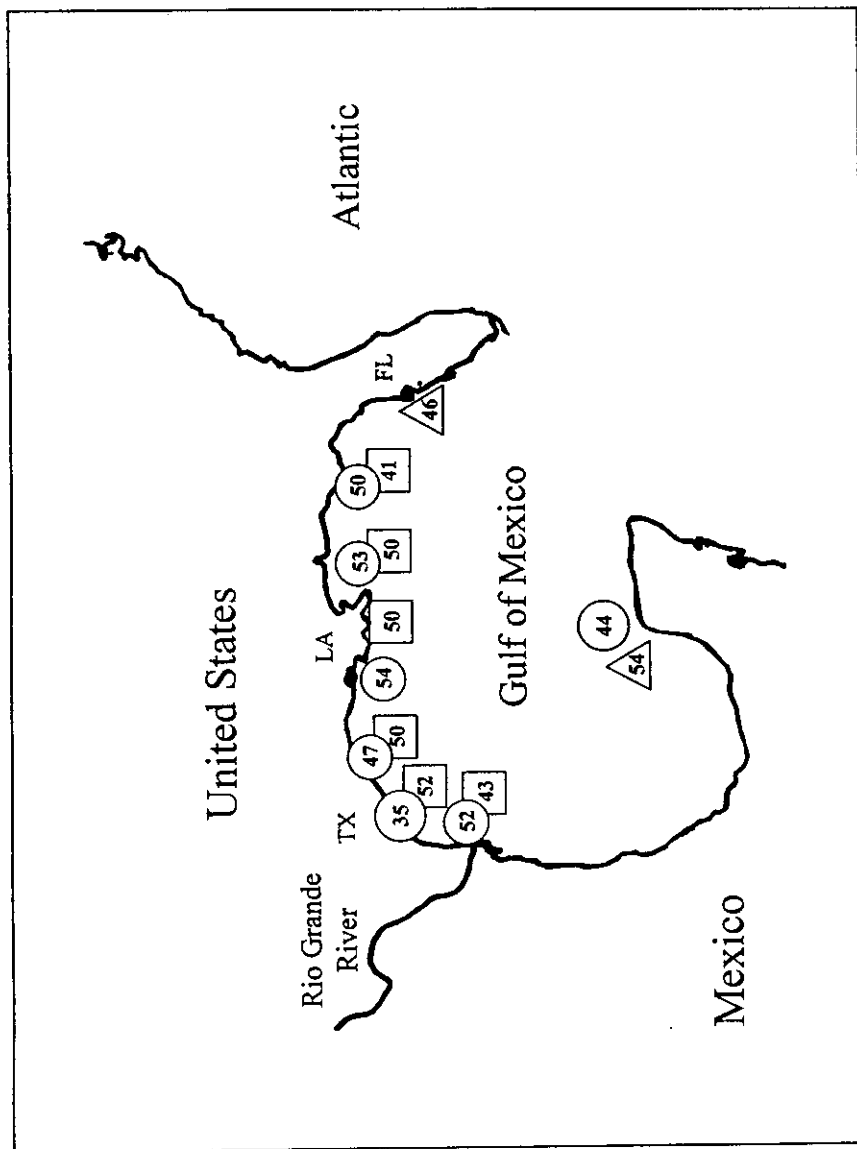
The second reason is the need for geographic definition when conducting stock assessments and allocations to resource users (Hilborn, 1985; Sinclair *et al.*, 1985).

In the following, we synopsise our current studies on variation in mitochondrial (mt)DNA of red snapper and red grouper from the Gulf of Mexico (Gulf). The studies are designed to determine whether discrete subpopulations (stocks) occur with either or both species. We have employed variation in mitochondrial (mt)DNA as the primary genetic tool to assess population structure for a number of reasons. Briefly, mtDNA is a genetically haploid, maternally inherited, cytoplasmic DNA molecule that in most cases is essentially identical in size and sequence in single individuals. The consequence of genetic haploidy and matrilineal inheritance is that mtDNA is expected to be four times more sensitive than nuclear-encoded genes in assessing the genetic impact of population subdivision (Templeton, 1987; Birky *et al.*, 1989). MtDNA also appears to have a more rapid rate of sequence evolution than nuclear-encoded genes (Brown, 1983; Wilson *et al.*, 1985), meaning that mtDNA should be useful in identifying subpopulations (stocks) of recent origin. The use of mtDNA to assess population structure is well documented (Awise, 1987; Moritz *et al.*, 1987; Ovenden, 1990).

#### MATERIALS AND METHODS

Samples of red snapper were procured from eight localities across the northern Gulf and from the Campeche Banks in Mexican waters (Figure 1). At six of the localities, samples were procured in subsequent years. Samples of red grouper were procured from the Florida Middle Grounds and from the Campeche Banks (Figure 1). Specific localities, numbers of individuals sampled at each locality, and methods of capture are described in the primary papers (red snapper - Camper *et al.*, 1993, Gold *et al.*, 1997; red grouper - Richardson and Gold, 1993, 1997). Tissues (generally heart and muscle) were removed from individual specimens, frozen in liquid nitrogen or freezers, and transported to College Station where they were stored at  $-80^{\circ}\text{C}$  until used.

We assayed mtDNA restriction-enzyme sites in each species following methods outlined in Gold and Richardson (1991). Specific restriction enzymes employed in each species are in the primary papers. MtDNA probes used for hybridization of Southern blots were the entire mtDNA molecules of each species cloned (separately) into bacteriophage ( and labeled with  $^{32}\text{P}$ -dCTP or  $^{32}\text{P}$ -dATP by random priming. MtDNA restriction sites in red snapper were mapped (Kristmundsdóttir *et al.*, 1996); restriction sites in red grouper were inferred from restriction fragment patterns. Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy *et al.*, (1992).



**Figure 1.** Localities for samples of red snapper and red grouper from the Gulf of Mexico and western Atlantic. Sample sizes are indicated within circles (red snapper procured in 1991), squares (red snapper procured in 1992), and triangles (red grouper).

Homogeneity of mtDNA haplotype frequencies between or among samples was assessed by a randomization (bootstrap) procedure (Roff and Bentzen, 1989). Significance levels for multiple tests performed simultaneously were adjusted by the sequential Bonferroni approach (Rice, 1989).  $F_{ST} (\Theta)$  values, a measure of the variance in mtDNA haplotype frequencies, were estimated following Weir and Cockerham (1984).

Phylogeographic structuring of haplotypes was examined by constructing minimum-length parsimony networks where individual haplotypes were connected in increments of (inferred) single restriction-site gains and losses. The spatial distribution of mtDNA haplotypes in red snapper was assessed via spatial autocorrelation analysis (SAAP; Wartenberg, 1989) to determine whether haplotype frequencies at a locality were independent of haplotype frequencies at neighboring localities. Low frequency haplotypes (those occurring in less than nine individuals) were removed to minimize "noise." Haplotypes used in SAAP runs are listed in Gold *et al.* (1997).

Within-sample mtDNA variability was assessed via (i) genotypic or nucleon diversity (probability that any two individuals drawn at random from a sample will differ in mtDNA haplotype), and (ii) intrapopulation nucleotide sequence or mtDNA diversity (average nucleotide sequence difference between any two individuals drawn at random). Both parameters were estimated by using equations in Nei and Tajima (1981). Bootstrapping, with 100 replicates per sample (Weir, 1990), was used to generate standard errors of nucleotide sequence (mtDNA) diversity in red snapper. Homogeneity among bootstrap-generated means was tested by using both parametric (Sokal and Rohlf, 1966) and non-parametric (Siegel, 1956) analysis of variance.

## RESULTS

Summary mtDNA data are given in Table 1. As all restriction enzymes used recognized six-base signals, approximately 560 base pairs were surveyed in both species, representing roughly 3.3% of the mtDNA genome. MtDNA nucleon and nucleotide sequence diversities differed trenchantly between the species, with estimates for red grouper being among the lowest reported for a marine fish species (see below).

Tests of homogeneity of mtDNA haplotype frequencies among samples of red snapper collected in each of two years were non-significant (Table 2), as were tests between samples collected in different years at the same locality (data not shown). V-tests (DeSalle *et al.*, 1987), employed to test homogeneity of frequencies of individual haplotypes (occurring in four or more individuals), also were non-significant (data not shown). The test for homogeneity of haplotype frequencies between the two samples of red grouper was non-significant as well (Table 2).

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**Table 1.** Summary of mitochondrial DNA variation: red snapper and red grouper.

Parameter	Red snapper	Red grouper
Number of individuals	707	100
Number of mtDNA restriction sites	93	93
Number of mtDNA haplotypes	92	16
Nucleon diversity	0.74	0.39
Nucleotide sequence diversity (In %)	0.22	0.06

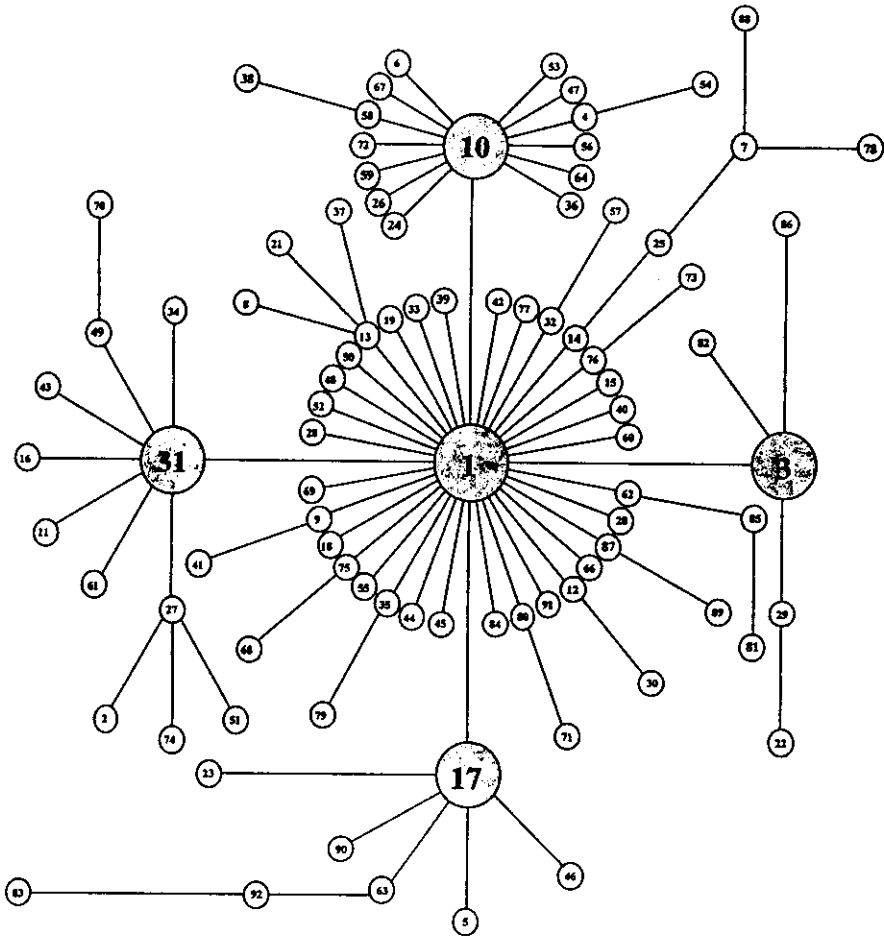
F<sub>ST</sub> ( $\Theta$ ) values (Table 2) corroborated homogeneity tests, as none of the F<sub>ST</sub> values differed significantly from zero.

**Table 2.** Tests of spatial homogeneity in mitochondrial DNA haplotype frequencies.

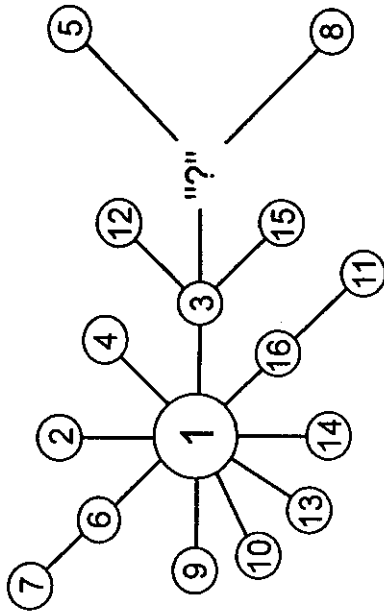
Test group	Number of samples	Number of haplotypes	<sup>a</sup> P <sub>RB</sub>	F <sub>ST</sub>
<b>Red snapper:</b>				
1991 collections	7	52	0.187	-0.004
1992 collections	6	49	0.324	-0.002
<b>Red grouper:</b>	2	16	0.550	-0.007

<sup>a</sup>P<sub>RB</sub>: Probability from randomization (bootstrap) approach of Roff and Bentzen (1989).

Minimum-length parsimony networks of individual haplotypes (Figures 2 and 3) revealed no evidence of phylogeographic structure (geographic partitioning) of individual haplotypes or haplotype lineages in either species.



**Figure 2.** Minimum-length parsimony network of mtDNA haplotypes of red snapper sampled from the Gulf of Mexico. Branch lengths are proportional to the number of (inferred) restriction-site changes (one or two) between haplotypes except for "hub" haplotypes (shaded) that differ from haplotype 1 by one restriction site.



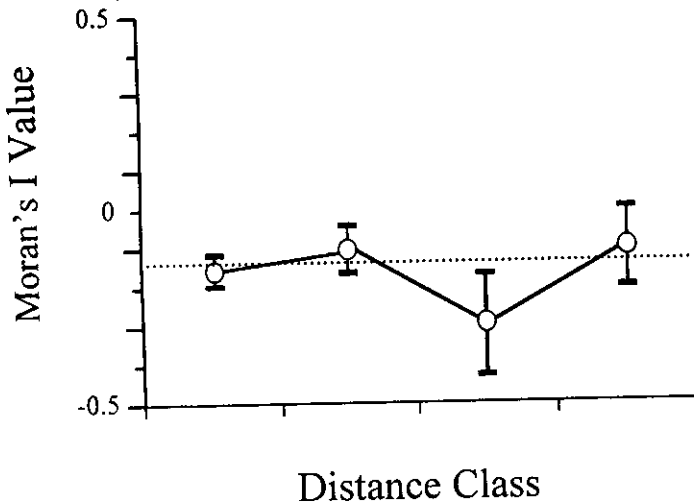
Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Florida	34	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1
Mexico	43	1	3	1							2	2	2	1	1	1

**Figure 3.** Minimum-length parsimony network of mtDNA haplotypes of red grouper sampled from the Gulf of Mexico. Branch lengths are proportional to the number of (inferred) restriction-site changes (one or two) between haplotypes. The haplotype designated "?" refers to a haplotype assumed to exist but not detected in this study.



Common haplotypes were found at all or nearly all sample localities, and haplotype groupings ("hubs") inferred from minimum-length parsimony networks were not restricted geographically. The absence of geographic cohesion of related haplotypes is best exemplified in red snapper where virtually all of the low-frequency haplotypes occur in two or more localities and are not restricted to two or three geographically contiguous localities (Table 3). Spatial autocorrelation analysis of common haplotypes in red snapper revealed no pattern of autocorrelation as a function of distance; mean autocorrelation coefficients (Moran's I values) were negative in all distance classes and did not differ significantly from values expected in the absence of autocorrelation (Figure 4).

A comparison of estimates of mtDNA nucleotide sequence diversity for various species of marine fishes in the Gulf of Mexico and western Atlantic is given in Table 4. Estimates for red snapper and (especially) red grouper are at the lower end of the spectrum. Because levels of mtDNA diversity are correlated with evolutionary-effective population size of females (Avisé *et al.*, 1988), the low values in red snapper and red grouper suggest historical and/or recent population bottlenecks (Bowen and Avisé, 1990; O'Brien *et al.*, 1987) where effective (female) population size was reduced.



**Figure 4.** Correlogram based on spatial autocorrelation analysis of mtDNA haplotype frequencies in samples of red snapper from the Gulf of Mexico. Abscissa: distance classes (left to right) based on equal frequencies per distance class. Ordinate: mean autocorrelation coefficients (Moran's I values) for each distance class. Bars about each mean represent one standard error on either side of a mean. Dashed line represents expected values when no correlation exists.

Table 3. Distribution of low-frequency mitochondrial DNA haplotypes in red snapper.

Sample locality	Mitochondrial DNA haplotype													
	4	6	7	13	14	15	17	19	27	29	30	38	55	68
Merida, Mexico	1	—	1	—	—	—	—	1	—	1	—	—	—	—
Port Isabel, TX	—	3	1	3	1	1	—	1	3	—	1	1	—	—
Port Aransas, TX	1	—	1	3	2	1	—	1	2	2	—	1	4	1
Galveston, TX	—	4	1	—	—	1	1	—	4	1	2	—	—	1
W. Cameron, LA	3	—	—	1	—	—	2	—	1	1	—	—	—	2
Grand Isle, LA	—	—	—	—	—	1	5	1	1	1	1	—	—	—
Mobile, AL	1	—	2	3	—	1	3	—	1	—	—	1	1	—
Pensacola, FL	1	1	1	1	1	—	—	—	—	—	—	—	—	—
Panama City, FL	3	1	3	1	1	1	6	1	—	1	—	1	1	1

**Table 4.** MtDNA nucleotide sequence diversity in marine fishes from the Gulf of Mexico and western Atlantic.

<b>Species</b>	<b>Number of Individuals Surveyed</b>	<b>Number of mtDNA haplotypes</b>	<b>Nucleotide sequence diversity (%)</b>
Bluefish <sup>1</sup>	372	40	1.23
Atlantic herring <sup>2</sup>	69	26	1.09
Red drum <sup>3</sup>	869	118	0.57
Greater amberjack <sup>3</sup>	444	49	0.55
Spanish sardine <sup>4</sup>	73	24	0.52
Black drum <sup>3</sup>	300	37	0.48
King mackerel <sup>3</sup>	678	122	0.47
Spotted seatrout <sup>3</sup>	470	81	0.45
Red snapper	707	92	0.22
Weakfish <sup>5</sup>	370	11	0.13
Red grouper	100	16	0.06
Common snook <sup>6</sup>	156	33	0.05
Atlantic sea bass <sup>7</sup>	19	3	0.03
Gulf black sea bass <sup>6</sup>	9	2	0.03

<sup>1</sup>Graves *et al.* (1992a); <sup>2</sup>Kornfield and Bogdanowicz (1987); <sup>3</sup>Gold and Richardson (1998); <sup>4</sup>Tringali and Wilson (1993); <sup>5</sup>Graves *et al.* (1992b); <sup>6</sup>Tringali and Bert (1996); and <sup>7</sup>Avisé (1992).

#### DISCUSSION

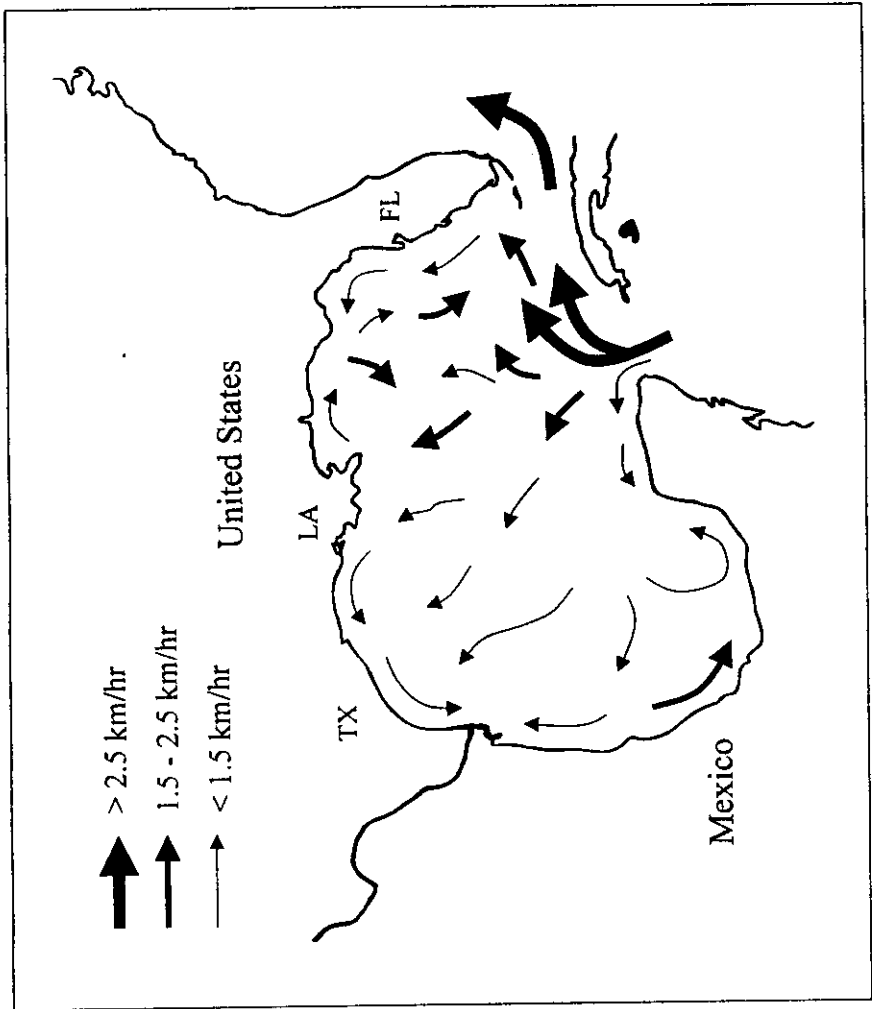
Genetic homogeneity and absence of spatial patterns in allele or haplotype distribution among geographic samples of a species are generally interpreted as evidence for a single population with no major barriers to gene flow (Scoles and Graves 1993; Graves *et al.*, 1992a,b; Baker *et al.*, 1995). Patterns of mtDNA variation in both red snapper and red grouper are thus consistent with the interpretation that each is comprised of a single population (stock) in the Gulf of Mexico. In both species, mtDNA haplotype frequencies were homogeneous among geographic samples and no phylogeographic structuring of haplotypes or haplotype lineages was evident. In red snapper, low frequency haplotypes were not clustered spatially and frequencies of common haplotypes were not correlated with distance. For red snapper, the "population" would extend minimally from the Campeche Banks to west Florida (the limits of our sampling). The red grouper population would include individuals from the Florida Middle grounds and the Campeche Banks.

Life-history information, movement patterns, and other information related

to the potential for gene flow are not especially well documented in either species, especially red grouper. Both are managed as "reef fish" (GMFMC 1989, 1991) that are associated with high- or low relief bottom, including reefs, rock outcrops, ledges, caves, and shipwrecks (Moe, 1969; Bradley and Bryan, 1975). Red snapper spawn offshore and release highly pelagic eggs and larvae that settle after 28 - 30 days (Leis, 1987). Mark-recapture and ultrasonic-tracking experiments generally indicate that post-larval red snapper are sedentary and essentially non-migratory (Beaumariage, 1969; Fable, 1980; Szedelmayr, 1997; Szedelmayr and Shipp, 1994), although some movement of individual adults across considerable distances is documented (Beaumariage and Wittich, 1966) and seasonal inshore/offshore movements may occur (Bradley and Bryan, 1975). Studies on the biology of red grouper are even fewer. Juveniles are thought to be sedentary, preferring to hide in crevices or shells; adults are members of the benthic community and occupy ledges and caverns formed by limestone reefs (Moe, 1969). Based on observations of related species (Mito *et al.*, 1967), the pelagic larval stage of red grouper could be 30 - 40 days. The pelagic eggs and larvae and the length of larval life in both species leads to the simple hypothesis that gene flow in both species is accomplished by hydrodynamic (planktonic) transport.

Caveats or cautions to this hypothesis are several-fold. First, as acknowledged by most authors, one cannot prove a null hypothesis. The spatial genetic homogeneity observed in both species is merely consistent with the notion that samples are drawn from a population with the same parametric haplotype frequencies. Further study with larger sample sizes might test the null hypothesis more rigorously, but at this point we would recommend that such study employ nuclear DNA markers that are more rapidly evolving than mtDNA (e.g. a microsatellite loci). A second caveat is that neither egg/larval type (i.e. pelagic or benthic) nor length of larval life is necessarily an effective predictor of gene flow in reef fish populations (Shulman and Bermingham, 1995). Actual egg/larval movements may be constrained by currents or life histories that have evolved to restrict larvae to natal areas (Johannes, 1978; Leis and Miller, 1976; Shulman and Bermingham, 1995). Surface current patterns in the Gulf of Mexico (Figure 5), for example, would seem to preclude unrestricted, two-way transport of eggs and larvae between the Florida Middle Grounds and the Campeche Banks. The relatively strong loop current that passes between the Yucatan Peninsula and Cuba before turning westward through the Florida Straits might assist egg/larval transport from the Campeche Banks northward, perhaps to the Florida Middle Grounds, but not the reverse. This would be especially critical in assessing gene flow/population structure in red grouper, as movement of post-larval (and benthic) red grouper between the Florida Middle Grounds and the Campeche Banks seems especially unlikely given the 100 - 2000 fathom

depths (Rezak *et al.*, 1985) that passage across the Florida Straits would necessitate. The loop current also should impede unrestricted movement of red snapper between the Campeche Banks and west Florida. However, red snapper (unlike red grouper) are common in the western Gulf where surface currents are not so directional or strong (Figure 5).



**Figure 5.** Surface currents in the Gulf of Mexico. Adapted from Shulman and Bermingham

A third caveat, at least in red snapper, is that the absence of spatial autocorrelation of common haplotype frequencies, in concert with spatial genetic homogeneity, suggests the (seemingly) unlikely notion that dispersal (gene flow) between geographically-extreme localities (e.g. south Texas and northwest Florida) is as likely as dispersal between any two, geographically contiguous localities. Even considering the 28 - 30 day larval period in red snapper, a "stepping-stone" pattern where movement is greater between spatially adjacent localities seems intuitive. Such a pattern is expected to lead to an "isolation-by-distance" effect where positive autocorrelation is observed in proximal distance classes and negative autocorrelation is observed in distal distance classes (Sokal and Oden, 1978). The absence of such a pattern in red snapper is perplexing.

A fourth caveat is that the observed genetic homogeneity may reflect past rather than present-day circumstances. Populations in either or both species could be isolated at least partially today yet have been in sufficient genetic contact in the recent (evolutionary) past to remain indistinguishable in haplotype frequencies and spatial patterning. We have suggested elsewhere (Richardson and Gold, 1997; Gold *et al.*, 1997) that colonization of newly available habitat in the northern Gulf following the last glacial retreat could have produced such a scenario in both species. Future studies that employ nuclear DNA markers that are more rapidly evolving than mtDNA (e.g. microsatellite loci) might effectively test this hypothesis as well.

A final comment regards the low levels of mtDNA variability within samples of both species, especially red grouper. The maternal mtDNA lineages in both species are less divergent in nucleotide sequence than mtDNA lineages observed in other marine fishes (Table 4) and in various freshwater fish species distributed across the southern United States (Bermingham and Avise, 1986; Richardson and Gold, 1995 a,b). The limited genetic divergence observed in red snapper and red grouper is consistent with the notion that high rates of mtDNA lineage extinction occurs (or has occurred) in both species. Rapid mtDNA lineage extinction could stem from reductions in effective (female) population size (genetic bottlenecks) or from a high variance in (female) reproductive success (Avise *et al.*, 1984; Hedgecock, 1994). The former (genetic bottlenecks) could have occurred historically during Pleistocene glaciations, more recently from overfishing or habitat deterioration, or both. The latter would reflect situations where large variation in larval mortality could occur across years resulting in considerable among-year variation in recruitment and situations where a small number of genetically related individuals could replace entire populations or subpopulations (Hedgecock, 1994). There also is the possibility in red grouper that the sexual system (asynchronous hermaphroditism) may affect both effective (female) population size and variance in (female) reproductive

success. Other asynchronous hermaphrodites (viz., black sea bass and common snook) also have extremely low within-sample mtDNA nucleotide sequence diversity (Table 4), and it may prove worthwhile to consider models as to how sexual mode might impact genetic diversity.

#### ACKNOWLEDGMENTS

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