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Population Structure and Variation in Red Snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic Coast of Florida as Determined from Mitochondrial DNA Control Region Sequence

Amber F. Garber,[1] Michael D. Tringali,[2] and Kenneth C. Stuck[1]

[1]*University of Southern Mississippi, College of Marine Sciences, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, MS 39566-7000, USA*
[2]*Florida Marine Research Institute, 100 Eighth Avenue SE, St. Petersburg, FL 33701-5020, USA*

Abstract: The mitochondrial DNA control regions of red snapper (*Lutjanus campechanus*) from the Gulf of Mexico (n = 140) and Atlantic coast of Florida (n = 35) were sequenced to generate a prestocking genetic baseline for planned stock enhancement. Intrasample haplotype and nucleotide diversities ranged from 0.94 to 1.00 and 1.8% to 2.5%, respectively. All population analyses were consistent with the hypothesis that red snapper constitute a single, panmictic population over the sampled range. A ubiquitous, predominant haplotype, shared by 23% of the specimens, appeared to be evolutionarily recent, in contrast to previous findings based on restriction fragment length polymorphism data. Tajima's D values were suggestive of a recent bottleneck. Mismatch distributions from Gulf samples were smooth and unimodal, characteristic of recent population expansion. However, the Atlantic sample exhibited a comparatively broader, possibly multimodal distribution, suggestive of a more stable population history. Additional control-region data may clarify potentially disparate demographic histories of Gulf and Atlantic snapper.

Key words: red snapper, *Lutjanus campechanus*, mtDNA control region, population structure.

INTRODUCTION

Red snapper are distributed from the Yucatán Peninsula, Mexico, throughout the Gulf of Mexico, and along the Atlantic coast, rarely occurring north of Cape Hatteras, North Carolina (Nelson and Manooch, 1982). These bottom-dwelling, carnivorous fish exist primarily over live corals and rocky bottoms at depths of 7 to 146 m (Camber, 1955; Moseley, 1966). Red snapper school a meter or so from the bottom, but can occur in surface waters (Camber, 1955). They are believed to inhabit a limited home range for considerable lengths of time (Camber, 1955). Adult snappers are reported to congregate in areas of hard limestone bottoms and irregular bottom formations (Camber, 1955; Moseley, 1966). However, analysis, of

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Current address for Amber F. Garber: North Carolina State University, Department of Zoology, Campus Box 7617, Raleigh, NC 27695-7617, USA.
Corresponding author: Amber F. Garber; e-mail afgarber@unity.ncsu.edu

stomach content indicates they are probably not confined to reefs, rocky areas, or other hard substrates, which is further supported by large commercial catches over offshore muddy bottoms (Moseley, 1966).

The Florida red snapper fishery began about 1850 off Pensacola (Camber, 1955), and by 1874 red snapper had become a market commodity in New York and Washington, D.C. (Goodyear, 1995). The commercial catch of red snapper declined continuously from 1945 to 1952, although the efficiency of the Pensacola fleet had increased, and there was no evidence of increased efforts by other fleets in that region (Camber, 1955). This decline could have resulted from a natural fluctuation in abundance. However, the fishery also may have been undergoing a decline wherein total catch decreased at a rate greater than the fishing effort (Camber, 1955). Twenty years later, Bradley and Bryan (1975) reported seafood dealers and fishermen were anxious over the decline of the fishery. Commercial and recreational red snapper fisheries are among the most important fisheries in the Gulf of Mexico; thus, overfishing became a major concern of both scientists and fishermen.

In response to grave concerns about declining fisheries in general, the federal government enacted the Magnuson Fishery Conservation and Management Act on April 13, 1976. Eight regional Fishery Management Councils (FMCs) were created to develop fishery management plans (FMPs). The Gulf of Mexico FMC (GMFMC) was charged to oversee fisheries in federal waters of the Gulf of Mexico. The GMFMC's Reef Fish FMP became effective November 1, 1984. Prior to its development, state regulations had had a negligible effect on conservation of reef fish fisheries (Goodyear, 1995).

Despite changes in management of its commercial and recreational sectors, the red snapper fishery has experienced major catch fluctuations, along with a decrease from historic fishery landings (Schirripa, 1998). It is thought that the decline in commercial fisheries, at least before the fishery was regulated, was due to high incidental takes of mainly juvenile fish by shrimp trawlers (Bradley and Bryan, 1975). Also, Futch and Bruger (1976) reported a large proportion of the total red snapper catch, both commercial and recreational, consisted of fish that had not reached sexual maturity. Various regulations have been amended and implemented for red snapper fishery management to allow rebuilding of populations. To limit the take of red snapper, regulations have utilized commercial quotas, seasonal closures, size requirements, bag limits (for recreational fishermen, daily catch limits), licensing strategies, and bycatch reduction devices. Still, total allowable catch (TAC) limits established by federal fishery regulations for adult red snapper have been exceeded (GMFMC, 1998; Schirripa, 1998).

Fishery managers have 3 strategies available to them to restore or replenish collapsed fish stocks: (1) regulate the fishing effort, (2) attempt to restore the degraded nursery and spawning habitats, and (3) enhance stocks via captive breeding and release (Munro and Bell, 1997). Strategies 1 and 2 are traditional management techniques employed singularly or in tandem. Strategy 3, stock enhancement, exists as an alternative when traditional management techniques have proved unsuccessful. Stock enhancement can be used as an option when the estimated recovery target dates (according to the spawning potential ratios, SPRs) for the stock are continuously projected further into the future. This is precisely the case with red snapper. Amendment I of the Reef Fish FMP defines a stock as overfished or in an overfished condition when it is below the level of 20% SPR (GMFMC, 1998). The Reef Fish FMP calls for 20% transitional SPR of these stocks within 1.5 generation times. To increase the SPR, there must be a decrease in fishing pressure in the directed fishery (adults) or decreased mortality caused by other means (i.e., bycatch juvenile mortality caused by shrimp trawling). In the case of red snapper, it has not been economically possible to decrease the yearly TAC, bycatch reduction devices have not decreased juvenile mortality enough, and additional biological information has revealed a longer life span and generation time. The date when the red snapper fishery would obtain 20% SPR was set at 2007 in 1991, 2009 in 1992, and 2019 in 1995 (GMFMC, 1998). With the reevaluation of 20% SPR dates further into the future, stock enhancement may become a feasible and necessary option for reestablishing the fishery.

The U.S. Gulf of Mexico Marine Stock Enhancement Program, for which the red snapper is the primary species of interest, acknowledges that hatchery-reared fish released into a wild population may have some genetic effect. The program is taking a responsible approach to stock enhancement from the onset and is making efforts to prevent deleterious effects by the involvement of various scientific disciplines. A stock enhancement program is considered effective when released cultured fish contribute reproductively (and thus genetically) to the target wild conspecifics. This reproductive contribution, however, should be achieved without significantly altering the genetic structure of the existing wild population. Because generational contributions cannot be traced with physical tagging, a reliable

genetic marker must be employed to monitor the integration of cultured fish into wild stocks.

From prior studies based on allozymes (Johnson, 1987), mitochondrial DNA restriction fragment length polymorphisms (RFLPs) (Gold and Richardson, 1994, 1998; Gold et al., 1997), and microsatellites (Heist and Gold, 2000; Gold et al., 2001), it is thought that red snapper comprise a single, panmictic population in the northern Gulf of Mexico. However, Chapman et al. (1996) identified variation among red snapper from 3 sampling sites in the northern Gulf of Mexico using 4 base restriction enzymes. In this study we have utilized a highly variable segment in the mtDNA control region to assess whether or not stock structure is apparent between various sampling sites in the Gulf of Mexico and Atlantic coast of Florida.

The control region was utilized because it is 4 times more discerning than nuclear-encoded genes when determining the genetic impact of population subdivision (reducing effective population size and increasing sensitivity to genetic drift) as a result of haploidy and maternal inheritance (Templeton, 1987; Birky et al., 1989). Further, the vertebrate mitochondrial genome accumulates mutations at a rate up to 10 times faster than single copy nuclear DNA on average (Meyer, 1993). Because it is noncoding, the control region accumulates mutations 2 to 5 times faster than the rest of the mitochondrial genome (Meyer, 1993). This increased rate of sequence evolution suggests utility in identifying subpopulations (stocks) of recent origin (Gold and Richardson, 1998). For example, Rosel and Block (1996) observed enough variation in a 300-bp segment of the control region to indicate swordfish populations were structured on a global scale. Reeb et al. (2000) identified population structuring of swordfish in the Pacific by sequencing a 629-bp portion of the control region. Seyoum et al. (2000) observed sufficient variability in a 369-bp portion of the control region in red drum to conclude that fisheries in the Atlantic and Gulf should continue to be managed separately.

A.F. Garber (2001) found that the majority of variation in red snapper was partitioned in a region (hypervariable region) flanked by conserved sequence, i.e., amenable to construction of PCR primers. Moreover, the segment that included the hypervariable region was sufficiently short to ensure complete overlap during double-stranded direct sequencing, reducing the possibility of sequencing errors. The high variability within a small mtDNA segment can work as an uncomplicated but powerful tool for future monitoring activities. Here, we assess the utility of the hypervariable mtDNA segment as a genetic marker by examining sequence variation among red snapper samples from geographically remote locations with specific focus on a potential stock enhancement release site (FH-1) off the Mississippi coast.

Materials and Methods

Red snapper were collected from the following coastal waters from 1998 to 2001 (Figure 1): Cancun, Mexico (CM, $n = 13$); Louisiana, west of the Mississippi River (LA, $n = 6$); release site Fish Haven-1 (FH-1, $n = 102$); Mississippi (MS, $n = 13$); Alabama (AL, $n = 6$); and the Atlantic coast of Florida (FL, $n = 35$). Grey snapper (*L. griseus*, $n = 14$), collected off Tampa Bay, Florida, in 1999, served as outgroup specimens. White muscle tissue or fin clips were excised from sampled fish and stored in SED buffer (saturated NaCl; 250 mM EDTA, pH 7.5; 20% DMSO) for later processing.

Total genomic DNA was extracted from each sample, using a modified phenol-chloroform isolation procedure (see N.M. Garber, 1999) or using the Puregene DNA Isolation Kit (Gentra Systems, Inc.). Puregene extractions were performed according to the manufacturer's directions except that the RNase A step was omitted. DNA was quantified using fluorescence spectrophotometry, as described by Gallagher (1994), with bisbenzimidazole (Hoechst no. 33258) and herring sperm DNA as the standard. Sample concentrations were adjusted to 100 ng/μl using 1 mM Tris and stored at −20°C if extracted with the phenol-chloroform protocol. DNA samples isolated with the Puregene Kit were diluted 1:5 with sterile water, and 2 μl of the dilution was used to seed a 50-μl PCR.

Primers A and G (Lee et al., 1995) were used to clone and sequence the entire control regions of 27 red snapper (FL = 8, LA = 6, MS = 13) and identify a hypervariable region (A.F. Garber, 2001). Red-snapper-specific PCR primers were constructed based on the conserved regions flanking the hypervariable region with M13 tails (M13 tails in bold; Invitrogen/Life Technologies) for direct sequencing: SNAPHYPM13F (5′-**CACGA CGTTG TAAAA CGACC** ACTTT CATCG ACGCT TGCA-3′) and SNAPHYPM13R (5′-**GGATA ACAAT TTCAC ACAGG** GTGAT CTTAG GAGTA TAGGG-3′). PCR amplification of the hypervariable region was performed in replicate 25-μl reactions containing 50 ng of template DNA or 2 μl of dilution, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.3 μM of each primer, and 1.75 units of *Taq* DNA

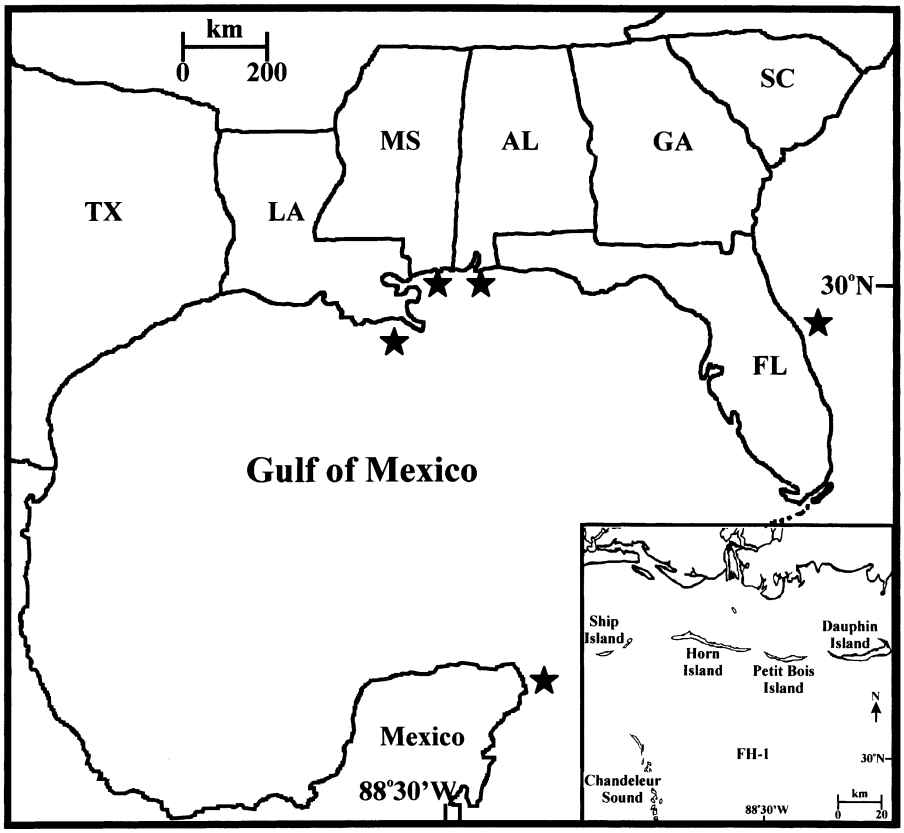

Figure 1. Red snapper (*Lutjanus campechanus*) sampling sites off Alabama, Cancun (Mexico), Florida Atlantic coast, Louisiana (west of the Mississippi River), and Mississippi. Inset at bottom right shows the approximate location of Fish Haven-1 (FH-1) at latitude 30°03′ 568–611 and longitude 88°36′ 544–576.

polymerase with 10× PCR buffer (Amersham Life Science). PCR cycling parameters consisted of an initial denaturation of 3 minutes at 94°C, followed by 35 cycles of 45 seconds at 94°C, 1 minute at 52°C, and 2 minutes at 72°C. A final elongation was carried out for 7 minutes at 72°C. PCR products were separated on a 1% agarose–TBE gel with ethidium bromide, and desired bands were visualized on a UV light box, excised, gel-purified (QIAGEN QIAquick Gel Extraction Kit), and quantified.

Gel medium was prepared according to the manufacturer's recommendations using KB^{PLUS} 5.5% Gel Matrix (LI-COR Biotechnology Division). Thermo Sequenase DYEnamic Direct cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech Inc.) and stop solution from Sequi Therm EXCEL II DNA Sequencing Kit (Epicentre Technologies) were employed for sequencing reactions, following the Thermo Sequenase Labeled Primer Cycle Sequencing protocol (LI-COR 4200 Series Sequencing Manual) with SBS modifications. Reactions were optimized utilizing 40 ng of template DNA in the sequencing reaction. PCR cycling reaction was an initial denaturation of 2 minutes at 95°C, and followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 62°C, and 30 seconds at 70°C. When the cycling program was completed, 2.0 μl of stop solution was added to each tube, and samples were denatured at 95°C for 2 minutes. For each completed sequencing reaction, 0.6 μl was loaded onto a 25-cm-long, 2-mm-deep polyacrylamide gel on a LI-COR model 4200 automated DNA sequencer and assayed. BaseImagIR Version 4.0 (Data Collection, Image Analysis, and SCF Utilities) was used to collect sequencing data from the gel. Sequences were edited using the program AlignIR Version 1.1 and visually reviewed.

Sequences were aligned using CLUSTALX Version 1.8 (Thompson et al., 1997) with the transversion-to-transition ratio weighted at 0.20 and the gap opening penalty increased to 20.00. Nucleotide composition was measured using ARELEQUIN Version 2.0b1 (Schneider et al., 1999). Alpha (α) was calculated for the entire data set by constructing the most parsimonious trees (PAUP* Version 4.0b4a; Swofford, 2000), the number of substitutions required at each site to create the most parsimonious tree was tabulated with a frequency distribution provided in MacClade (Maddison and Maddison, 1992), and this distribution was entered into GAMMA (Sullivan et al., 1995).

Genetic diversity was measured by haplotype diversity (h) and nucleotide diversity (π, based on unweighted pairwise differences between haplotypes). Genetic distances

Table 1. Measures of Genetic Diversity at Six Red Snapper (*Lutjanus campechanus*) Sampling Locations

Population[a]	Sample size	No. of haplotypes	Percentage occurrence[b]	Molecular Diversity Indices	
				Haplotype (h)	Nucleotide (π)
AL	6	6	0.167	1.000 ± 0.096	0.024 ± 0.015
CM	13	11	0.231	0.962 ± 0.050	0.018 ± 0.011
FH-1	102	70	0.245	0.940 ± 0.020	0.020 ± 0.011
FL	35	25	0.257	0.936 ± 0.035	0.025 ± 0.013
LA	6	6	0.167	1.000 ± 0.096	0.019 ± 0.012
MS	13	13	0.077	1.000 ± 0.030	0.022 ± 0.013
All sequences	175	109	0.229	0.946 ± 0.014	0.021 ± 0.011

[a]Abbreviations for sample locations are given in the text. [b]Percentage occurrence of the predominant haplotype in each sample location.

were calculated in ARLEQUIN Version 2.0b1 with the γ-corrected ($\alpha = 0.136603$) substitution model of Tamura and Nei (1993; hereafter Tamura-Nei). Diversity measures were computed for the following geographic groupings: the entire data set, AL, CM, FH-1, FL, LA, and MS. Tajima's test of selective neutrality or Tajima's D (Tajima, 1989; Proseq Version 2.9b; Filatov, 2001) was conducted to determine if sequence substitutions occurred in accordance with expectations for neutral mutation; negative D values may indicate marker nonneutrality or that a population has experienced a bottleneck. To examine phylogeography in red snapper, a neighbor-joining tree based on Tamura-Nei distances between haplotypes was constructed using MEGA Version 2.1 (Kumar et al., 2001) and rooted by using grey snapper haplotypes. The pairwise-deletion option for nucleotide insertions and deletions was invoked.

Geographic structure was analyzed as follows. Analysis of molecular variance (AMOVA) was used to partition genetic variance within and between groups of samples (ARLEQUIN). AMOVA analyses, based on Tamura-Nei distances, were performed by comparing samples from all localities with no additional hierarchy (AL, CM, FH-1, FL, LA, MS) and by grouping Gulf samples (AL, CM, FH-1, LA, MS) and Atlantic samples (FL only). An exact R × C test (ARLEQUIN; 100,000-step Markov chain, 3000 dememorization steps) was used to test for differences in haplotype distributions among locations (Raymond and Rousset, 1995; Goudet et al., 1996); test criteria for table-wide significance were adjusted using the sequential Bonferroni technique (Rice, 1989). Finally, population pairwise F_{ST} values were calculated using ARLEQUIN (Tamura-Nei; γ correction; 1000 permutations) to generate a distance matrix using samples grouped by location (AL, CM, FH-1, FL, LA, MS).

Population demographic history was examined for evidence of a recent bottleneck by generating mismatch distributions (Li, 1977; Harpending, 1994; Rogers, 1995) in ARLEQUIN using uncorrected Tamura-Nei distances. The observed frequencies for the number of substitutions between pairs of individuals and expected (model) frequencies were graphed using Sigmaplot 2000 (SPSS Inc.). Demographic parameters τ, θ_0, and θ_1 (and their associated SSD P values) were estimated using a nonlinear least-squares approach (Schneider and Excoffier, 1999). Harpending's (1994) raggedness index was computed for each distribution, and its significance was tested using a parametric bootstrap approach (1000 replicates) in ARLEQUIN.

Results

Sequences of the hypervariable region for 175 red snapper from AL, CM, FH-1, FL, LA, and MS ranging from 287 to 293 bp were aligned to produce a 297-bp consensus sequence (GenBank accession numbers AF356881–7004, AF356750–776, and AY153500–23). Average nucleotide composition was 20.82% C, 32.13% T, 26.19% A, and 20.86% G. There were 66 polymorphic sites, including 49 transitions, 11 transversions, and 19 indels. Among the 175 red snapper sequenced, 109 different haplotypes were present. Eighty-nine of the red snapper haplotypes (51%) occurred as singletons. Twenty haplotypes were shared by more than one fish. One of the shared haplotypes was common to 40 of the red snapper sequenced and present in fish from all 6 sampling sites (Table 1). For each sample, the sequence similarity ranged from 91% to 100%.

Red snapper exhibited high haplotype and nucleotide diversity over the hypervariable region at each of the 6

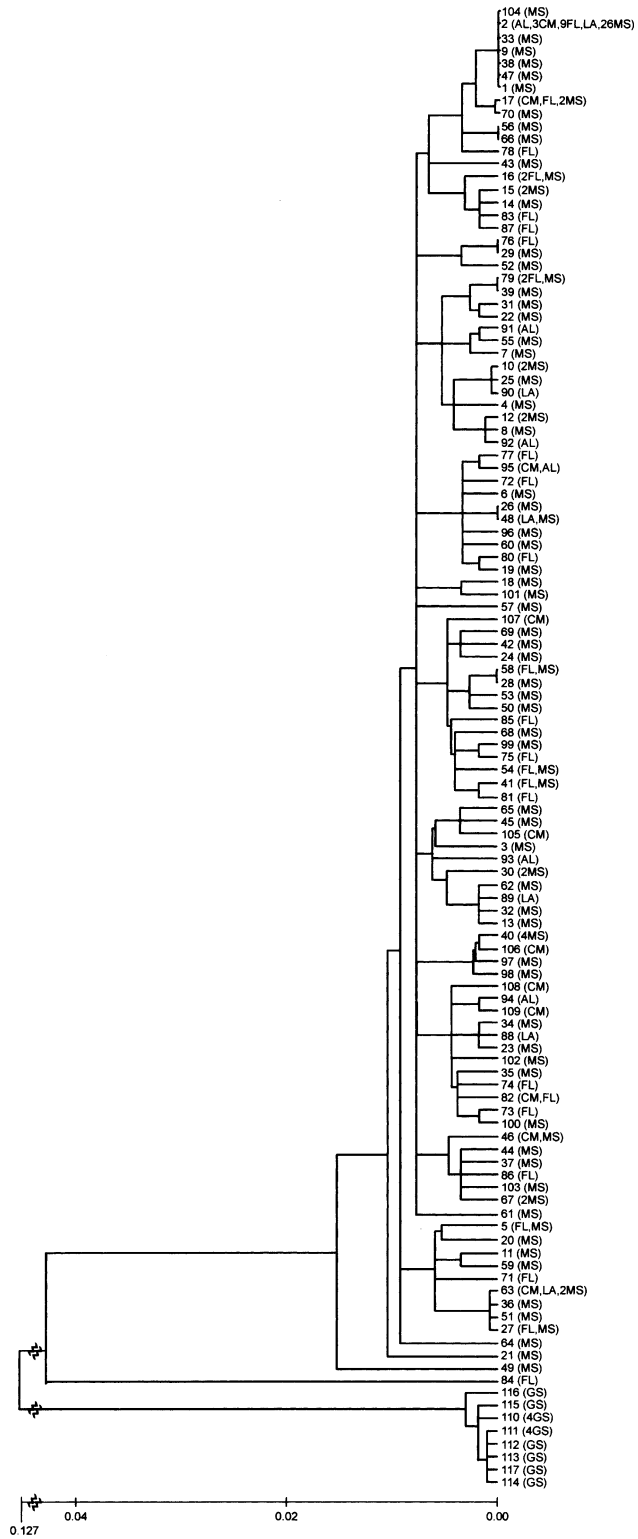

Figure 2. Rectangular linearized neighbor-joining tree depicting relatedness of red snapper (*Lutjanus campechanus*) haplotypes and rooted by grey snapper (*L. griseus*) haplotypes. To the right of each haplotype number and in parentheses are the numbers of red snapper and sampling locations: Alabama (AL, $n = 6$); Cancun, Mexico (CM, $n = 13$); Florida Atlantic coast (FL, $n = 35$); Louisiana (LA, $n = 6$); and Mississippi (MS, $n = 115$) with the exception of grey snapper (GS). Scale shows branch lengths.

sampling locations (Table 1). Indices of haplotype diversity reached a maximum of 1.0 in the AL, LA, and MS locations (possibly because of small sample sizes). Levels of nucleotide diversity ranged from a low of 0.018 in the CM site to a high of 0.025 in the FL site ($\pi = 0.021$ overall).

Tajima's D was negative and significant for all locations (entire data set = −1.9037, AL = −0.6968, CM = −0.2942, FH-1 = −1.2755, FL = −1.3229, LA = −0.6015, and MS = −0.5029). A negative Tajima's D is indicative of a marker that is nonneutral or that the population may have undergone a bottleneck. If a bottleneck was recent, a star-like phylogeny may be observed (Tajima, 1989) in an interhaplotype tree. To explore this, a neighbor-joining tree was constructed that included grey snapper sequences (GenBank accession numbers AY245003–16) to root the tree (Figure 2). This tree showed no evidence of phylogeographic structuring among samples from various geographic areas, and the predominant haplotype occurred in a relatively derived position. Moreover, all computed molecular variance (AMOVA) was contained within samples when considering each sampling site separately (100.76%, Table 2a) and when considering samples in Gulf and Atlantic groups (100.30%, Table 2b). The R × C test displayed nonrandom distribution of haplotypes between some pairs of samples; P values ranged from 0.00 to 1.00 (Table 3). From significance in corrected P values, distinctions could be made between FH-1 and FL and FH-1 and MS (lack of differentiation among other samples could have been due to small sample sizes). Population pairwise F_{ST} values were low or negative (Table 3), indicative of a population having high overall gene flow. Interestingly, the highest value of F_{ST} was observed between the 2 sites in Mississippi (MS and FH-1).

Mismatch distributions were graphed for the entire group of sequences and separately for 3 groups (Figure 3). These mismatch distributions were unimodal and smooth (with the exception of FL), which would indicate that since the time when mitochondrial haplotypes coalesced, the population has grown rapidly. Harpending's raggedness index ranged from 0.01 to 0.11 (Table 4) with no significant P values (note that FL $P = 0.0610$); low index numbers are indicative of a smooth mismatch distribution, i.e., an expanding population. The variance in the mismatch distribution of the Florida sample was considerably higher than

Table 2a. Analysis of Molecular Variance of Red Snapper (*Lutjanus campechanus*) Mitochondrial DNA Hypervariable Region Sequences Separated into Six Locations: AL (n = 6), CM (n = 13), FH-1 (n = 102), FL (n = 35), LA (n = 6), MS (n = 6)

Source or variation	*df*	Sum of squares	Variance components	Percentage or variation
Among populations	5	14.495	−0.02585 Va	−0.76
Within populations	169	582.730	3.44811 Vb	100.76
Total	174	597.225	3.42226	

Table 2b. Analysis of Molecular Variance of Red Snapper (*Lutjanus campechanus*) Mitochondrial DNA Hypervariable Region Sequences Separated into Two Locations: Gulf (n = 140), Atlantic (n = 35)

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among populations	1	2.863	−0.01023 Va	−0.30
Within populations	173	594.362	3.43562 Vb	100.30
Total	174	597.225	3.42539	

Table 3. The R × C Exact Test of Population Differentiation or a Test of Haplotype Frequencies Was Calculated Providing Nondifferentiation Exact P Values for Six Geographical Groupings of Red Snapper (*Lutjanus campechanus*) Found to the Lower Left of the Diagonal

Population pairwise F_{ST} values

	FH-1	FL	LA	AL	MS	CM
FH-1		−0.004	0.001	−0.023	0.026	−0.023
FL	0.000 (0.000)*		−0.013	−0.037	0.003	−0.037
LA	0.044 (0.015)	0.109 (0.012)		−0.085	−0.069	−0.031
AL	0.024 (0.007)	0.121 (0.023)	1.000 (0.000)		−0.055	−0.091
MS	0.002 (0.001)*	0.013 (0.006)	1.000 (0.000)	1.000 (0.000)		−0.016
CM	0.005 (0.003)	0.004 (0.001)	0.316 (0.010)	0.335 (0.010)	0.218 (0.008)	

Nondifferentiation exact P values

[a]An * denotes significant P values following correction for multiple tests. Pairwise F_{ST} values are located to the upper right of the diagonal.

the mean (Table 4), a condition not shared by the distributions of other samples.

Discussion

The occurrence of 2 or more highly variable nucleotide segments flanked by conserved (invariable) segments is a hallmark of the mtDNA control region in animals, including many fish species (Lee et al., 1995; Rosel and Block, 1996). The location of the region of highest variability, however, differs among fish species. In the swordfish *Xiphius gladius* (Rosel and Block, 1996) and the striped mullet *Mugil cephalus* (N.M. Garber, 1999), the DNA segment of greatest variability was located near the 5′ end of the control region, whereas in 8 species of anadromous Pacific salmonids *Oncorhynchus*, the Atlantic salmon *Salmo salar*, and the Arctic grayling *Thymallus arcticus* (Shedlock et al., 1992), this segment was identified at the 3′ end. The segment of highest variability in red snapper was near the 3′ end of the control region (A.F. Garber, 2001) and termed the hypervariable region.

Sequence diversity in the mtDNA control region was relatively high in red snapper. In this study 51% of the specimens had unique haplotypes, and one haplotype was shared by 23% of those sequenced. In a previous study

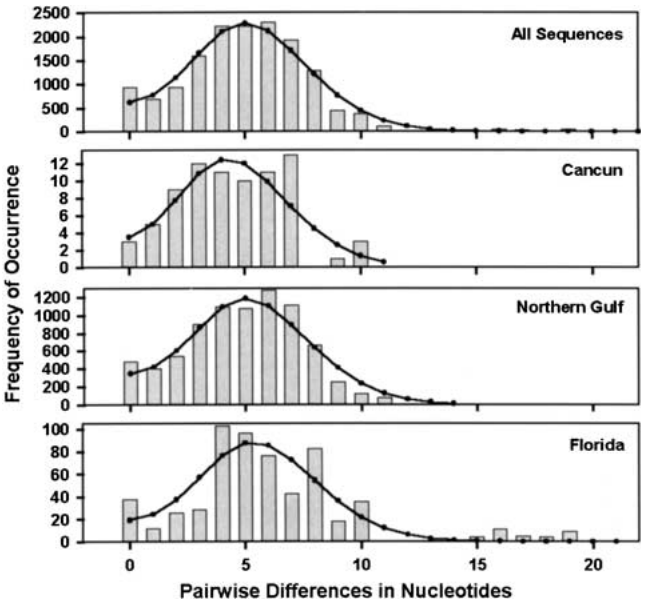

Figure 3. Mismatch distributions based on control-region sequences of red snapper (*Lutjanus campechanus*) from various sampling sites. Samples sizes for these distributions varied: All Sequences = 175; Cancun, Mexico = 13; Northern Gulf (AL, LA, MS) = 127; Florida = 35. Linear plots represent the expected distributions for a population that has undergone demographic expansion (Rogers and Harpending, 1992; Rogers, 1995).

based on mtDNA (genome-wide) RFLPs, Gold et al. (1997) reported a lower percentage of unique red snapper haplotypes (8%) and a much higher percentage of the most commonly shared haplotype (48%). The occurrence of numerous, unique control-region haplotypes was reflected in the high haplotype and nucleotide diversities observed here (Table 1). Indeed, haplotype and nucleotide diversities in this study were higher (h = 0.95; π = 2.1%) than those in several previous RFLP studies of red snapper (h = 0.74 to 0.78 and π = 0.22% to 0.50%: Gold and Richardson, 1994, 1998). Whereas differences among the red snapper studies were likely due to methodological differences, the diversities observed in this study were comparable to those based on sequences of a 369-bp portion of the 3′ control-region segment in the red drum *Sciaenops ocellatus*, where h = 0.98 and π = 3.0% (Seyoum et al., 2000). In general, the pattern of diversity in the red snapper control region conforms to the "shallow" evolutionary pattern described for fishes by Shields and Gust (1995) and Grant and Bowen (1998)—i.e., numerous rare haplotypes differing from a single or few prevalent haplotypes by 1 or 2 mutations.

On the basis of prior mtDNA RFLP and microsatellite studies (Gold and Richardson, 1994; Gold et al., 1997, 2001; Heist and Gold, 2000), red snapper in the northern Gulf of Mexico are thought to constitute a single, panmictic population. The mtDNA sequence data in this study were generally consistent with that hypothesis—a predominant haplotype was geographically ubiquitous and the remaining were not phylogeographically structured, F_{ST} values were low and insignificant, and all of the molecular variance was due to within-sample (as opposed to between-sample) differences. In an RFLP analysis of 707 Gulf of Mexico red snapper, Gold and Richardson (1998) identified a single, numerically dominant, putatively ancestral haplotype. Such a haplotype would be shared by all populations and might dominate genetic analyses; thus, its distribution would reflect evolutionary history and potentially confound estimates of current gene flow. In contrast, the predominant haplotype identified in this study was not basally located. Its relatively distal position suggests that it may have arisen recently from an evolutionary perspective. Because it occurred in Gulf and Atlantic samples at approximately the same frequencies (Table 1), homogenizing levels of gene flow among these regions must have occurred since its inception, as supported by low F_{ST} values. Species of marine fish, having pelagic eggs, larvae, juveniles, or adults (high levels of dispersion), free of physical geographic barriers, and exhibiting continuous distribution, are expected to have somewhat extensive and recent historical gene flow interconnections, resulting in limited population structure (Avise et al., 1987).

To date little is known of the demographic history of red snapper in the Gulf of Mexico. The significantly negative Tajima's D values observed here could be an indication that the population underwent a recent bottleneck. Further, the smooth, left-skewed shape and low mean and variance of the red snapper mismatch distribution are suggestive of a population that has recently expanded (Harpending, 1994); such distributions are inconsistent with those expected by stable populations at demographic equilibrium or historically isolated populations now in secondary contact. Although the Florida Atlantic sample was not significantly ragged (P = 0.0610), its mismatch distribution was more indicative of long-term stability. Also, a highly divergent, basal haplotype was observed among the Florida specimens, whereas such haplotypes were not observed among the Gulf red snapper. If red snapper in the Gulf of Mexico have undergone a bottleneck and recent expansion, then it is possible that red snapper from the Florida Atlantic coast, a putatively more stable assemblage, populated or repopulated the Gulf of Mexico

Table 4. Mismatch Distribution Results for Red Snapper (*Lutjanus campechanus*) from the Entire Sample and the Sample Separated into Six Locations According to Geographic Localities[a]

	Parameters							
Region	*n*	Mean	Variance	τ	θ_0	θ_1	Raggedness index	*P*
All sequences	175	5.056	7.903	5.757	0.002	25.161	0.0100	0.6930
AL	6	5.333	2.524	5.424	0.000	6657.500	0.1067	0.4890
CM	13	4.474	5.551	4.654	0.427	24.216	0.0360	0.6360
FH-1	102	4.847	6.472	5.961	0.001	20.317	0.0122	0.6190
FL	35	6.008	13.954	6.057	0.003	31.763	0.0440	0.0610
LA	6	4.067	5.924	1.722	2.756	6655.000	0.0711	0.8410
MS	13	5.244	5.433	4.206	1.106	6078.125	0.0204	0.8390

[a]The model of sudden demographic expansion parameters (Rogers and Harpending, 1992; Rogers, 1995) and Harpending's raggedness index (1994) are presented.

via immigration. Alternatively, it is possible that the Gulf population declined and recovered without significant immigration from the Atlantic. Based on similar shallow patterns in mtDNA genealogies, Grant and Bowen (1998) hypothesized that regional populations of sardines and anchovies were exposed to intermittent extinctions and recolonizations. It would be necessary to sequence more samples from the Atlantic coast of Florida to distinguish between these possibilities.

Szedlmayer (1997) used ultrasonic telemetry at artificial reef sites in the northeast Gulf of Mexico to show red snapper have limited movement and spend more time on artificial reefs than previously expected. A red snapper tagged by these researchers was recaptured at the release site 3.7 years later (Szedlmayer, 1997). If red snapper possess some site fidelity, it would be easier to monitor hatchery-reared and released individuals. Although gene flow estimates were high, significant R × C test *P* values between Florida and a Mississippi site (FH-1) and between the 2 Mississippi sites indicated that red snapper haplotypes may be nonrandomly distributed in some cases. Thus red snapper spawning and recruitment may be coupled to some degree. If mtDNA haplotype distributions are temporally stable within more restricted ranges, it may be possible to detect a significant hatchery input to FH-1 for stock enhancement purposes. A companion study of (prestocking) temporal mtDNA variance among 4 red snapper year-classes from the FH-1 site is in progress.

Overall, although sample sizes were limited in some cases, our data are consistent with the hypothesis of minimal, if any, geographic structuring of mtDNA diversity among red snapper in the Gulf of Mexico. This is important information for a stock enhancement program because there may be a lower risk of detrimental genetic impact when attempting to enhance a population of fish having limited genetic structure and high diversity. The high haplotype and nucleotide diversities observed here suggest that the hypervariable region might be useful as a genetic monitoring tool in stocked red snapper populations. In particular, these data provide a prestocking baseline that will be useful in tracking temporal genetic changes, should they occur, in stocked populations of northern Gulf red snapper. Finally, the question of whether red snapper from the Florida Atlantic coast exhibit a different demographic history than Gulf snapper will be left to future research efforts.

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References

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., and Saunders, N.C. (1987). Intraspecific

phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* 18:489–522.

Birky, C.W. Jr., Fuerst, P., and Maruyama, T. (1989). Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics* 121:613–627.

Bradley, E., and Bryan, C.E. (1975). Life history and fishery of the red snapper (*Lutjanus campechanus*) in the northwestern Gulf of Mexico: 1970–1974. In: *Proc. of the 27th Annual Gulf and Caribbean Fisheries Institute and 17th Annual International Game Fish Research Conference*, pp 77–106.

Camber, C.I. (1955). A survey of the red snapper fishery of the Gulf of Mexico, with special reference to the Campeche Banks. *Tech Ser Fla St Bd Conserv* 12:1–64.

Chapman, R.W., Bortone, S.A., and Woodley, C.M. (1996). A molecular approach to stock identification and recruitment patterns in red snapper (*Lutjanus campechanus*). In: *Biology, Fisheries and Culture of Tropical Groupers and Snappers*, Arreguín-Sánchez, F., Munro, J.L., Balgos, M.C., and Pauly, D. (Eds.). Makati City, Philippines: ICLARM, pp 85–91.

Filatov, D. (2001). Processor of Sequences Version 2.9b. University of Edinburgh. Available at *http://helios.bto.ed.ac.uk/evolgen/filatov/proseq.html*.

Futch, R.B., and Bruger, G.E. (1976). Age, growth, and reproduction of red snapper in Florida waters. *Florida Sea Grant College Program Report* 17:165–184.

Gallagher, S.R. (1994). Commonly used biochemical techniques, Appendix 3. In: *Current Protocols in Molecular Biology*, Janssen, K. (Ed.). New York, N.Y: John Wiley and Sons, Inc., pp A.3.10–A.3.11.

Garber, A.F. (2001). Utilization of a hypervariable region as a molecular marker for red snapper, *Lutjanus campechanus*, stock enhancement. M.Sc. thesis. University of Southern Mississippi, Hattiesburg.

Garber, N.M. (1999). Application of the mitochondrial DNA control region in population structure studies of *Mugil cephalus* (striped mullet) in North America. M.Sc. thesis. University of Southern Mississippi, Hattiesburg.

GMFMC (1998). Regulatory amendment to the Reef Fish Fishery Management Plan for red snapper including total allowable catch, bag limits, minimum size limits, and seasons. Tampa, Fla.: Gulf of Mexico Fishery Management Council, pp 1–57.

Gold, J.R., and Richardson, L.R. (1994). Mitochondrial DNA variation among 'red' fishes from the Gulf of Mexico. *Fish Res* 20:137–150.

Gold, J.R., and Richardson, L.R. (1998). Genetic homogeneity among geographic samples of snappers and groupers: evidence of continuous gene flow? *Proc Ann Gulf Carib Fish Inst* 50:709–726.

Gold, J.R., Sun, F., and Richardson, L.R. (1997). Population structure of the red snapper from the Gulf of Mexico as inferred from analysis of mitochondrial DNA. *Trans Am Fish Soc* 126:386–396.

Gold, J.R., Pak, E., and Richardson, L.R. (2001). Microsatellite variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. *Mar Biotechnol* 3:293–304.

Goodyear, C.P. (1995). Red snapper in U.S. waters of the Gulf of Mexico. Miami, Fla.: National Marine Fisheries Service, Southeast Fisheries Science Center, Miami Laboratory, MIA-95/96-05, pp 1–171.

Goudet, J., Raymond, M., de Meeüs, T., and Rousset, F. (1996). Testing differentiation in diploid populations. *Genetics* 144:1933–1940.

Grant, W.S., and Bowen, B.W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardine and anchovies and lessons for conservation. *J Hered* 89:415–426.

Harpending, R.C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol* 66:591–600.

Heist, E.J., and Gold, J.R. (2000). DNA microsatellite loci and genetic structure of red snapper in the Gulf of Mexico. *Trans Am Fish Soc* 129:469–475.

Johnson, A.G. (1987). An investigation of the biochemical and morphometric characteristics of red snapper (*Lutjanus campechanus*) from the southern United States. Panama City, Fla.: National Marine Fisheries Service, pp 1–8.

Kumar, S., Tamura, K., Jacobsen, I., and Nei, M. (2001). MEGA2.1: Molecular Evolutionary Genetics Analysis, Version 2.1. Pennsylvania State University, University Park, and Arizona State University, Tempe.

Lee, W., Conroy, J., Howell, W.H., and Kocher, T.D. (1995). Structure and evolution of teleost mitochondrial control regions. *J Mol Evol* 41:58–66.

Li, W.H. (1977). Distribution of nucleotide differences between two randomly chosen cistrons in a finite population. *Genetics* 85:331–337.

Maddison, W.P., and Maddison, D.R. (1992). MacClade Version 3: an analysis of phylogeny and character evolution. Sunderland, Mass: Sinauer Associates, Inc.

Meyer, A. (1993). Evolution of mitochondrial DNA in fishes. In: *Biochemistry and Molecular Biology of Fishes, Molecular Biology*

Frontiers,, Hochachka, P.W., and Mommsen, T.P. (Eds.). vol. 2, Amsterdam, The Netherlands: Elsevier Science Publishers, pp 1–38.

Moseley, F.N. (1966). Biology of the red snapper, *Lutjanus aya* Bloch, of the northwestern Gulf of Mexico. *Publ Inst Mar Sci Univ Tex* 11:90–101.

Munro, J.L., and Bell, J.D. (1997). Enhancement of marine fisheries resources. *Rev Fish Sci* 5:185–222.

Nelson, R.S., and Manooch, C.S. (1982). Growth and mortality of red snappers in the west-central Atlantic Ocean and northern Gulf of Mexico. *Trans Am Fish Soc* 111:465–475.

Raymond, M., and Rousset, R. (1995). An exact test for population differentiation. *Evolution* 49:1280–1283.

Reeb, C.A., Arcangeli, L., and Block, B.A. (2000). Structure and migration corridors in Pacific populations of the swordfish *Xiphius gladius*, as inferred through analyses of mitochondrial DNA. *Mar Biol* 136:1123–1131.

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

Rogers, A.R. (1995). Genetic evidence for a Pleistocene population explosion. *Evolution* 49:608–615.

Rogers, A.R., and Harpending, H.C. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* 9:552–569.

Rosel, P.E., and Block, B.A. (1996). Mitochondrial control region variability and global population structure in the swordfish, *Xiphius gladius*. *Mar Biol* 125:11–22.

Schirripa, M.J. (1998). Status of the red snapper in U.S. waters of the Gulf of Mexico: updated through 1997. Miami, Fla.: National Marine Fisheries Service, Southeast Fisheries Science Center, Miami Laboratory, SFD-97/98-30, pp 1–85.

Schneider, S., and Excoffier, L. (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152:1079–1089.

Schneider, S., Roessli, D., and Excoffier, L. (1999). ARLEQUIN Version 2.0: a software for population genetic data analysis. Geneva, Switzerland: Genetics and Biometry Lab, Department of Anthropology, University of Geneva.

Seyoum, S., Tringali, M.D., Bert, T.M., McElroy, D., and Stokes, R. (2000). An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences. *Fish Bull* 98:127–138.

Shedlock, A.M., Parker, J.D., Crispin, D.A., Pietsch, T.W., and Burmer, G.C. (1992). Evolution of the salmonid mitochondrial control region. *Mol Phylogenet Evol* 1:179–192.

Shields, G.F., and Gust, J.R. (1995). Lack of geographic structure in mitochondrial DNA sequences of Bering Sea walleye pollock, *Theragra chalcogramma*. *Mol Mar Biol Biotechnol* 4:69–82.

Sullivan, J., Holsinger, K.E., and Simon, C. (1995). Among-site rate variation and phylogenetic analysis of 12S rRNA in sigmodontine rodents. *Mol Biol Evol* 11:261–277.

Swofford, D.L. (2000). Phylogenetic analysis using Parsimony* (and other methods) Version 4.0b4a. Sunderland, Mass.: Sinauer Associates Inc.

Szedlmayer, S.T. (1997). Ultrasonic telemetry of red snapper, *Lutjanus campechanus*, at artificial reef sites in the northeast Gulf of Mexico. *Copeia* 4:846–850.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595.

Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10:512–526.

Templeton, A.R. (1987). Genetics systems and evolutionary rates.. In: *Rates of Evolution*, Campbell, K.F.S., and Day, M.F. (Eds.). Canberra: Australian Acad. Sci., pp 218–234.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997). The CLUSTALX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882.